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CHROMOSOMAL DEFICIENCIES AND THE EMBRYONIC DEVELOPMENT OF DROSOPHILA MELANOGASTER[1]

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Chromosome aberrations have marked effects upon the development of an organism. Many such aberrations are lethal. In *Drosophila* this is particularly true of chromosomal deficiencies, as first described by Bridges.[2] A more detailed study of the effects of such aberrations made by Li[3] shows that in all the cases he investigated deficiencies are lethal in the homozygous condition—the organisms dying in the egg or larval stages. Heterozygous deficiencies result in death in later stages, although many are not lethal. A few exceptional cases of viable homozygous deficiencies have been described.[4,5] In such instances the deficiencies are among the smallest known, probably for very few genes.

These facts emphasize the importance of the chromosomes in the developmental processes. Just what the chromosomal functions may be, however, is by no means clear; nor is the rôle of the individual gene evident. An ideal approach, such as the removal of one gene at a time, then of combinations of genes, to determine the part played by each and its interactions with others, presents many practical difficulties. The existence of numerous deficiencies, however, makes an approximation to this approach possible by the study of the effects of larger or smaller blocks of genes.

The present study is concerned with the effects of certain deficiencies upon the embryonic development of *D. melanogaster*. The deficiencies used involved greater or lesser portions of the *X*-chromosome, ranging from the total absence of the *X* to a small deficiency involving relatively few "bands" as seen in the salivary gland chromosome. The technique of Huettner and Rabinowitz[6] was used, in a somewhat modified form, for the observation of living eggs, and details of internal structure were studied by means of sectioned material. Only timed eggs were used. Usually females were allowed to lay for half an hour and the eggs allowed to develop to the desired stage at a temperature of 22–23°C.

Nullo-X Eggs: One-quarter of the eggs of $\widehat{XX}Y$ females fertilized by normal males receives no X-chromosomes. These YY or "nullo-X" eggs do not develop beyond the earliest stages. Observations on both living and sectioned and stained material show that cleavage proceeds in the absence of the X-chromosome. There is, however, no uniform distribution of the nuclei throughout the egg, and the normal migration of nuclei to the surface at the 256 nuclei stage does not occur.[7] Germ cells are rarely formed, for the nuclei seldom reach the polar region of the egg. Usually there are no nuclei in the posterior portion of the egg, which contains only coalesced protoplasm or yolk. The nuclei remain dividing in the anterior half. Between the fourth and fifth hours of development they form a more or less solid mass containing between 500 and 1000 nuclei. Cytoplasm does not collect around the nuclei and no cells are formed. Yolk spheres clump together in the central or posterior portions of the egg and become sharply separated from the material which would normally become cytoplasm. A few nuclei are usually included in this mass of cytoplasm. These are larger and less pycnotic than the other nuclei and seem to undergo fewer divisions.

In normal eggs the elapsed time up to the formation of the germ cells and the blastoderm is one hour and forty minutes at 24°C.,[8] and blastoderm formation is accomplished within two hours of fertilization at the temperature used (22–23°C.). The nullo-X eggs at this time contain nuclei only in the anterior half, chiefly in the region of the fusion nucleus. The separation of the yolk and cytoplasm begins at this time and is complete in six-hour eggs.

In eggs four to six hours old the nuclei are densely packed in the anterior portion of the egg. The nuclei are mostly of the same size, but scattered here and there are larger nuclei containing more chromatin. There is usually one such nucleus to every 15 to 20 smaller ones. Although the chromatin is usually clumped so that no chromosome counts can be made, the number of ends which appear in the larger nuclei may be several times that of the smaller nuclei. This indicates polyploidy. Disintegration of nuclei sets in by the sixth hour.

Thus in the absence of the X-chromosome mitosis proceeds, but there is no regular migration of nuclei. Formation of cells and subsequent morphogenesis fail.

Half-X Eggs: Eggs deficient for half of the X-chromosome were obtained by mating males carrying a translocation involving the X- and the fourth chromosomes to $\widehat{XX}Y$ females. In the translocation used, $T1$, 4-*CRB*, the X is broken near its genetic middle. One-half of the X (to the right of lozenge and including Bar) has been intercalated between the fourth chromosome and its spindle fibre, while the other half of the X including lozenge carries the X spindle fibre. The only zygotes from such a cross which fail

to develop beyond the egg stage are those deficient for the entire *X* or one or the other of its halves. The nullo-*X* eggs are readily recognizable as described above. There remain (if the extra *Y* chromosome is neglected) only two other types of deficient eggs: those deficient for the left half of *X*, and those deficient for the right half. These are readily distinguishable, at the time of blastoderm formation, from the normal and nullo-*X* eggs. But no distinction can be made between the two half-*X* deficiencies.

The nuclei migrate to the surface, so that for a time a single layer of nuclei can be found on the surface of the egg. These nuclei, however, continue dividing and do not accumulate cytoplasm and produce cells. Together with the blastema they form a syncytial mass. In living eggs the regular pattern, which begins to appear on the surface as the nuclei reach it, becomes very irregular and forms a striking contrast to that of a normal incipient blastoderm. Thus no blastoderm is ever formed and all subsequent development of the half-*X* eggs is abnormal. The nuclei continue to divide and come to lie four or five deep over the central contents of the egg, chiefly the yolk with some protoplasm. The clumping of yolk and the coalescing of cytoplasm characteristic of nullo-*X* eggs does not take place.

The distribution of the nuclei is fairly constant. In older eggs (10–16 hours) they continue to lie around the yolk entirely filling the region normally occupied by the germ band at the time of its greatest extension.[9] Division of the nuclei continues slowly in this period; only a few divide at a time. The nuclei continue even at this late stage to be fairly uniform in size. There are few polyploid nuclei. Nuclear degeneration has not set in. A few division figures were found in sections of eggs 16 hours old. Older eggs were not examined.

In a smaller proportion of these older eggs the beginning of a constriction or furrow around the anterior end can be seen in the position at which the cephalic furrow forms in the normal embryo during the third hour of development.[9] This is the only observational indication of two types of deficient eggs. It seems entirely possible that eggs lacking one of the half-*X* chromosomes exhibit the furrow, while those lacking the other half do not.

Thus it is seen that in the absence of either half of the *X*-chromosome development becomes abnormal because of the failure of cell formation and growth, and the subsequent failure to produce a blastoderm.

Notch-8 Eggs: Notch-8 is a deficiency extending from just to the left of the white locus (1.5) to, but not including, the locus of echinus (5.5) in the *X*-chromosome. Mohr[10] who found this deficiency was unable to demonstrate it cytologically, but studied it in considerable detail genetically. It has since been described in the salivary gland chromosomes by Mackensen.[11] One-fourth of the expected offspring of Notch-8 females fail to ap-

pear. These are the Notch-8 males which Li[3] found to die in the egg stage after some development had taken place.

Observations made on living eggs show that the pole cells and blastoderm form normally and that no externally apparent irregularities occur in the early stages. Hence normal and deficient eggs are indistinguishable until somewhat later. The most marked irregularity which is noted in the living egg appears in the middle period of development (11–12 hours) when the germ band normally contracts and the larval segmentation becomes pronounced.[9] Only the faintest signs of segmentation appear in the Notch eggs and contraction does not occur.

The details were followed in sections. The most striking feature of such eggs is that they contain very little or no endoderm or mesoderm. The proctodaeal and stomodaeal invaginations occur, the ventral blastoderm elongates, apparently without the formation of the underlying layers, and the embryo looks superficially normal. The yolk is never enclosed, for the mid-gut fails to form. The hind-gut and its diverticula, the Malpighian tubes, never attain their normal position. The development of the hind-gut is abnormal; it comes to fill a larger portion of the posterior part of the egg than normal. It is possible that some of this material represents endoderm. The fact that no endoderm can be found united with the stomodaeum, however, indicates that the process of germ layer formation has been interfered with seriously. Nothing which can be interpreted as mesoderm has been found in these embryos. The ectoderm proliferates especially along the ventral mid-line and produces what appears to be a semblance of the early nervous system. There is no very sharp demarcation between this nervous tissue and the outer ectoderm. The cells are more densely packed in this region; in the median part clear areas which resemble the nerve fibre portions of the normal nervous system become more prominent in 14- and 16-hour eggs. There is no shortening of the embryo and the hind-gut remains with its opening in the dorsal side. In eggs 20 to 22 hours old the abnormal development is still pronounced, but there is as yet no sign of disintegration or cellular breakdown. The ventral nervous tissue and the hind-gut remain the most characteristic features. In the case of normal eggs of this age the young larva is complete and actively moving preparatory to the escape from the egg membranes.

In the case of the Notch-8 male it is clear that while the missing part of the chromosome is not essential for the formation of cells and the production of a blastoderm, it must be present if the inner germ layers are to be produced normally and take part in subsequent morphogenesis.

From the evidence which has been presented it is clear that certain, if not most, sections of the *X*-chromosome are essential for specific processes in the embryonic development of *Drosophila*. The larger the section of the chromosome missing (i.e., the more genes missing) the earlier and more

general are the causes of abnormal development, while the smaller the missing piece the more specific the effects. Thus in the region at the left end of the *X*-chromosome Muller[4] has found "a viable two gene deficiency," and Demerec and Hoover[5] have found a deficiency which survives in homozygous condition. In this same region many small lethal deficiencies are known, one of which (Deficiency scute-8) has been found to cause death very late in the egg stage just before hatching (Poulson, unpublished). Also small lethal deficiencies are found scattered throughout the chromosome complement. Hence it would appear that genes which play very definite parts in the embryonic developmental processes are distributed quite as much at random throughout the *X*-chromosome as are genes which affect only the most superficial characters.

A more detailed account of the behavior of these deficiencies, together with some further data, will be presented elsewhere.

[1] Work carried out at the Wm. G. Kerckhoff Laboratories, California Institute of Technology, Pasadena.

[2] Bridges, C. B., *Genetics*, **2**, 445 (1917).

[3] Li, J. C., *Genetics*, **12**, 1 (1927).

[4] Muller, H. J., *Jour. Hered.*, **26**, 469 (1935).

[5] Demerec, M., and Hoover, Margaret E., *Jour. Hered.*, **27**, 206 (1936).

[6] Huettner, A. F., and Rabinowitz, Morris, *Science*, **78**, 367 (1933).

[7] Huettner, A. F., *Jour. Morph.*, **37**, 385 (1923).

[8] Howland, R. B., and Child, G. P., *Jour. Exp. Zoöl.*, **73**, 109 (1935).

[9] Poulson, D. F., "The Embryonic Development of *Drosophila melanogaster*," *Actualites Scientifique et Industrielle*, Paris. (In press.)

[10] Mohr, O. L., *Z. f. indukt. Abst. Vererbl.*, **32**, 108 (1923).

[11] Mackensen, O., *Jour. Hered.*, **26**, 163 (1935).

DEFICIENCIES AND STRUCTURAL VARIATIONS WITHIN THE GIANT CHROMOSOMES IN RELATION TO THE PROBLEM OF GENE STRUCTURE

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The giant chromosomes in salivary and certain other glands of dipteran larvae are characterized by transverse chromatic discs or "bands," separated from one another by intervening achromatic material. Since one of the primary aims of studies on these chromosomes is to identify the genic as distinguished from non-genic materials, and to interpret the visible structures in terms of individual genes, attention naturally becomes focused

on the two types of constituents and on the visible components of each. If one type of material is genic and the other non-genic it seems probable, on general grounds, that it is the chromatic which is genic,[1] although the opposite view is also supported.[2] In the present state of the evidence it seems best to consider that either or both may be genic. For this reason we have devoted much attention to the morphological characteristics of each. Some of the results of these observations are considered below.

In *Sciara*, as indicated previously,[3] it may readily be seen that, in fixed preparations at least, the achromatic material is in the form of droplets or "vacuoles" and is essentially discontinuous, while the chromatic material is not thus organized and is in this sense essentially continuous. Each two successive chromatic discs are separated by a layer or plate of achromatic droplets, and correspondingly each two successive transverse layers of achromatic droplets are separated by a disc. The thickness and conspicuousness of the disc depends on how much chromatin it contains. The thinner ones range down almost to the point of invisibility and may appear merely as the boundaries between layers or plates of droplets. Although confined mainly to the transverse discs, the chromatic material may, and apparently does, extend between and around the achromatic droplets separating one disc from the next. It may be considered to be continuous both longitudinally and transversely, forming the groundwork of an essentially honey-comb type of organization. Many detailed features are not yet understood, but it is believed that the general aspects of visible organization in these chromosomes may all be interpreted in terms of the distribution of the two types of materials just mentioned.

One of the puzzling aspects of the present problem has resulted from the fact that the detailed structural characteristics of individual chromosomes vary greatly. In different preparations, or even different cells in the same gland, wide differences in detail are found in a given region of any chromosome (see published photographs).[4] In some cases the transverse chromatic discs are clear cut, relatively smooth in outline and apparently dense (deeply staining), while the achromatic material between them is likewise sharply outlined and is almost transparent after ordinary staining methods. In other cases, however, the chromatic material is much more widely dispersed, and the achromatic droplets are very conspicuous because of the chromatin surrounding them. The chromosome is "vesiculated."

In vesiculated chromosomes, discs which are apparently single in the banded type often appear double, exhibiting a layer or plate of achromatic droplets between the two faces. This accounts in part for the frequent occurrence of paired discs. In extreme form the structures thus formed may appear inflated and be biscuit-like or capsule-like. Some discs tend to exhibit this characteristic under almost all conditions, while others show it

only when extreme vesiculation occurs.[5] At first it was thought that the vesiculated condition was abnormal and due to injury or methods of treatment; but the evidence now indicates that, although treatment may cause modifications, the entire range of conditions may occur and even be reversible in living, normal chromosomes. In larvae from a culture at one stage the chromosomes may be vesiculated, while in sisters taken at a later period they may be in a sharply "banded" condition. Sometimes both conditions are found simultaneously in one gland.

Study of individual discs and regions of a chromosome in the vesiculated and non-vesiculated conditions indicates that with an increase of achromatic material "within" the (now double) discs there is a decrease of achromatic material between the discs, and vice versa. In all cases the achromatic material appears in the form of droplets in fixed preparations. Thus it appears that the exact distribution of the two types of material is not fixed, and also that the achromatic droplets do not have special significance as individual structures.[6] The fundamental pattern represented by the chromatic discs appears to be fairly definite and regular, but many of the discs may appear as either single or double "bands," as indicated above, and their structural details, as well as those of the achromatic materials, exhibit wide variation. This variability applies also to the so-called "granules" of chromatic material which, like some of the heavy-walled droplets of achromatic material, have been considered by some observers to represent genes.[7]

As noted previously, the relationship between these two constituents indicates that the shape, size, number and position of the granules are determined by the droplets. The variability of the granules is illustrated by the fact that a disc which appears in the form of a single transverse row of granules under one condition (non-vesiculated) may exhibit two distinct rows under other conditions (vesiculated), even in the same gland. Likewise, the evidence indicates that some rows of granules are formed from materials derived from two separate discs and that a single disc may contribute to two separate rows of granules.[8] The granules appear to represent thickenings in the discs (where the latter are distorted by pressure of adjacent achromatic droplets) or accumulations of chromatin in spaces between the droplets.

In considering the distribution of the achromatic materials two questions immediately present themselves. If an apparently single disc may be transformed into a double disc by the acquisition of achromatic material between its two faces, it may be asked whether the original disc was really single or double. No criterion is at present available for distinguishing between single and double discs except appearance. So far as known any disc, even the thinnest, may become double, or form a "capsule," in this manner. This consideration raises the second question, namely, is there

any difference between achromatic materials inside a double disc or "capsule" and those outside or between successive discs or "capsules?" Special attention has been given to this feature. No distinction has been detected. The achromatic materials inside the paired discs or capsules apparently exhibit the same range of characteristics as do those outside or between them. In either case the droplets may be small or large, thin walled or thick walled, depending on the particular region in which they lie. Likewise, capsules may be formed between two dissimilar discs, or between two pairs of discs, as well as between the two faces of a double disc or the two members of a pair—i.e., capsules may be formed by achromatic materials in either type of position.

In the search for the essential unit of structure here especial interest attaches to small deficiencies. Several cases of what appear to be single disc deficiencies have been studied in *Sciara ocellaris*. In each a pair of discs or a capsule is present in one homologue, and a single disc, apparently corresponding to one member of the pair, is present in the other homologue. The achromatic material on either side of the single disc appears to correspond to that on the corresponding sides of the pair of discs in the other homologue. The deficient member thus appears to lack one disc and the achromatic material between the two discs of the other member. Apparently, therefore, the achromatic material on one side of a disc is associated with it, and acquired or lost with it, or else a regulating factor of some kind determines the general nature or amount of achromatic material on either side of a given disc. General considerations would make the latter interpretation appear the more probable.

As noted previously the achromatic droplets differ greatly in size, number and appearance in different regions of a given chromosome, and equally great differences are exhibited by the chromatic "granules." Since both types of structures have been considered by some authors to represent genes, brief consideration may be given here to their size characteristics and to certain relationships between them and the structures visible in chromosomes of ordinary embryonic cells such as the salivary gland cells are before their enormous enlargement takes place.

The view which regards the granules or heavy-walled droplets as representing individual genes is based on the stated or implied assumption that the genes and the chromonemata (individual series of chromomeres) have definite size limits, and that growth automatically results in duplication. On this view a giant chromosome is large because the original chromonema which gave rise to it has undergone repeated divisions, giving a multiple structure like a cable. The number of "granules" in a transverse disc, then, indicates the degree of multiplication, and each granule (or droplet, as the case may be) identifies an individual chromonema. One of the difficulties inherent in such a view is illustrated by the smaller cells

of the mature salivary gland. Some of these possess chromosomes which, although enlarged like the cells to many times their original size, nevertheless appear single. In a purely descriptive sense such a structure may be designated a single chromonema, but it bears little resemblance to a chromonema in an "ordinary" cell.

Two aspects of the problem may be emphasized here, one dealing with size and the other with the shape and general appearance of the structures under consideration. The apparently single chromonema in these smaller salivary gland cells is many times as large as a single chromonema in ordinary embryonic cells. Likewise, the individual droplets or granules, which form a single linear row here, are many times as large as any units (chromomeres) visible in chromonemata of ordinary cells. It seems evident, therefore, either that the components of a chromonema (including genes?) do not have fixed size limits corresponding to those seen in an ordinary cell, or else that the visible structures under consideration are not single.

The same two alternatives are met in any attempt to interpret the droplets and granules in fully developed giant chromosomes. If either a droplet or a granule is considered to represent an individual gene the gene evidently does not have a fixed size limit that is reached in an ordinary cell. If either is considered to represent a gene with surrounding accessory material, the size limits of chromonemata and chromomeres similarly are not fixed and size is no necessary indication of the constitution of a chromosome in terms of chromonemata. Likewise there is no fixed relation between number of chromonemata or chromosomes and size of cell or nucleus, and it must be assumed that the bulk of a chromonema is composed of non-genic material produced by the genes or having the power of independent growth. Size of components in a chromonema, then, would have no obvious significance in relation to the problem of the physical nature of genes. There would be little theoretical difference between such a view and one which assumed the entire giant chromosome to be a single, enlarged chromonema.

On the other hand, if each droplet or granule is considered to be compound and to represent a bundle of sister genes, derived by multiplication of the original one, then the group of droplets or granules at any one locus (disc or band) presumably represents a group of sisters derived by multiplication of the original droplet or granule—which was itself compound. Thus a multiple would itself become a unit and undergo division when it reached a certain size. Unsupported by direct evidence such an assumption appears merely to complicate the situation.

Concerning the shape and general appearance of the structures under consideration it is to be observed that visible evidence from the smaller salivary gland cells, as well as general considerations, indicate that a chro-

monema is not to be regarded as a series of chromomeres on a thread, like a string of beads, but as a structure of more uniform diameter and more like a slender cylinder. This consideration seems important, because, on the view which regards the giant chromosome as a cable composed of visible chromonemata, the granulated striations seen when the chromosome is stretched are considered to represent these chromonemata. In the striations the granules or heavy-walled droplets appear like beads on a string. Likewise, the longitudinal lines between successive chromatic discs in the undistorted chromosome are considered on this view to represent the threads connecting successive granules or chromomeres. On our view these lines represent the interfaces, together with more or less chromatic material, between the achromatic droplets separating one chromatic disc from the next, and the striations in stretched chromosomes represent distortions of this fundamental honey-comb-like structure.[9] If numerous chromonemata are present in the giant chromosomes we would not expect them to show the characteristics of these lines or striations, but rather to appear as slender cylinders, more like the fibrils of a striated muscle fibre. And, as indicated in earlier papers, if chromonemata of the type found in ordinary embryonic cells are present here their number should be very large and they should be practically invisible as units.

[1] See, e.g., Caspersson, T., *Skandinav. Arch. f. Physiol.*, Supplement to **73**, 1–151 (1936).

[2] Koltzoff, N., *Science*, **80**, 312 (1934).

[3] Metz and Gay, these PROCEEDINGS, **20**, 617–621 (1934); Metz, *Jour. Hered.*, **26**, 177–188 (1935 *a*); Metz, *Jour. Hered.*, **26**, 491–501 (1935 *b*); Metz, *Biol. Bull.*, **71**, 238–248 (1936).

[4] Figures 4, 5, 6, Metz, 1935 *a*, loc. cit.

[5] In certain regions of some chromosomes the materials regularly tend to appear in the form of heavy-walled droplets or as clusters of bubbles. These apparently represent two or more independent discs, with intervening achromatic materials—the chromatic material in this case being dispersed around the periphery of the droplets. Examples are shown by Bauer (*Zoöl. Jahrb.*, **56**, 239, 1936, figure 6) and by the writer (1935 *a*, loc. cit., figure 5B, at end of chromosome).

[6] According to observations of Dr. D. F. Poulson and the writer an excess of achromatic material in a given region may result in the formation of a nucleolus. The chromatic discs may extend out into the nucleolus in the form of a branching root-like system. The expanded regions or "puffs" (see, e.g., photographs in figure 5B, C, Metz 1935 *b*, loc. cit.) are thus to be regarded as essentially nucleolar in nature.

[7] See, e.g., Koltzoff, N., loc. cit.; Bridges, C. B., *Amer. Nat.*, **69**, 59 (1935); Koller, P. C., *Proc. Roy. Soc. B.*, **118**, 371–397 (1935); Bauer, H., *Zeit. Zellforsch.*, **23**, 280–313 (1935) and loc. cit.

[8] Metz, 1935 *b*, loc. cit., and unpublished observations.

[9] As noted elsewhere, our observations on *Sciara*, *Chironomus* and *Drosophila* indicate that these striations or lines are not continuous or thread-like in nature, but form a branching meshwork or basketwork at all levels unless the chromosome is so severely distorted as to disorganize it. In other respects also conditions in *Chironomus* and *Drosophila* agree with those in *Sciara*.

DEVELOPMENT OF EYE COLORS IN DROSOPHILA: EXTRACTION OF THE DIFFUSIBLE SUBSTANCES CONCERNED

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Introduction.—The development of eye color in Drosophila is known to involve special diffusible substances.[1,2] A genetically vermilion (*v*) eye will develop wild-type eye color if it is supplied with v^+ substance by transplantation or by injection of body fluid of wild type flies. Similarly a genetically cinnabar (*cn*) eye will develop the color characteristic of wild type if it is supplied with cn^+ substance. The present paper summarizes preliminary experiments made to learn something of the nature of the two substances just mentioned.

During the course of our studies, Ephrussi and Harnly[3] have shown that pupal fluid can be freed of living cells by freezing in liquid air without destroying the v^+ and cn^+ substances. Khouvine, Ephrussi and Harnly[4] have shown further that these substances can be extracted from Calliphora pupae with 95 per cent alcohol-ether mixtures and with 95 per cent alcohol but not with pure ether. They conclude that these substances are not proteins or enzymes, a conclusion confirmed by our work.

Method of Testing for the Substances.—Liquid extracts are tested for v^+ substance by injections of approximately 0.4 cubic mm. into the body cavities of apricot vermilion (*w^a v*) or vermilion brown (*v; bw*) larvae shortly prior to puparium formation. In a similar way, cn^+ substance is tested for by injections into cinnabar brown (*cn bw*) larvae. The test flies normally have practically colorless eyes, and the presence of an active substance is indicated by a darkening of the eye color. The maximum modification results in an eye color similar to apricot or brown, depending on the constitution of the test fly.

It was found desirable to measure responses in a quantitative way. The test flies were separated into groups of increasing intensities of eye color and these groups were assigned values from 0 to 5. The value 0 represents no response and 5 a response such that the color is indistinguishable from brown control flies. The mean of the values obtained in this way, using from 10 to 20 injected flies, thus gives a figure which expresses in a rough quantitative way the intensity of the reaction. It is known from Malpighian tube-transplants[5] that the response varies with the number of tubes implanted and therefore presumably with the quantity of substance available in the test animal. It is not assumed that the difference in concentrations of active substance measured by any unit difference in the arbitrary scale is the same. Indeed the results suggest that a considerably

greater increase in concentration is represented by the difference between 4 and 5 than by that between 0 and 1.

Preliminary Experiments.—Wild-type pupae of *D. melanogaster*, 24 to 60 hours after puparium formation, were crushed and centrifuged into three layers, an upper one of foam or oil, a second of clear yellowish liquid and a bottom layer of sludge made up largely of cells and pupa cases. The aqueous layer, injected into w^a *v* larvae, produced a very faint or doubtful intensification in the color of the eyes; the other layers were completely negative. On the assumption that the substances were being inactivated by oxidation, pupae were crushed under sesame oil. Fluids obtained in this way were negative in all tests. The oil layers were likewise consistently negative even when the volume of oil was kept small to avoid dilution. A chloroform extract of the pupae, taken up in sesame oil, was also negative. It appeared that the substances were not lipoid-soluble, and therefore attention was re-directed to the preparation of water extracts. It was soon found that if extracting and centrifuging were carried out in an atmosphere of nitrogen, a clear watery layer could be obtained which gave strong positive tests for both v^+ and cn^+ substances.

Extractions in Nitrogen.—The procedure finally evolved was to use a wide tube, drawn out at the base into a thin-walled capillary. At the point where the bore narrowed a slight constriction was made by thickening the glass, and on this was rested a pad of glass wool. The pupae were put into the wide tube, and a piece of narrow glass tubing, sealed at one end, but with a small hole blown in the side of the seal, was fitted into the wide tube by means of rubber sleeves. Nitrogen was passed in through this tube for some minutes, the capillary then sealed off and the pupae thoroughly crushed in the atmosphere of nitrogen. The crusher was finally disconnected from the nitrogen supply and quickly sealed off, and the whole tube was then centrifuged at about 2500 r. p. m. for 15 minutes. The glass wool acts as a coarse filter, retaining pupa cases, and a clear yellowish aqueous layer collects in the capillary.

The fluid so obtained—about 0.1 to 0.2 cc. from 300 pupae—gave positive results of a strength about 2.5 in *v; bw* and from 1.5 to 2.0 in *cn bw*. The fluid prepared in this way showed a strong precipitate of coagulable protein on heating in boiling water. Heating the vessel containing pupae in boiling water for 2–3 minutes, before crushing and extraction, however, did not affect the results. Subsequent heating of the fluid extracted from heated pupae produced no further precipitation. The substances are therefore extremely heat-stable, and hence are not to be classed with enzymes or true proteins. Further, if the juice, extracted in nitrogen from boiled pupae, was exposed to air for 24 hours and then injected, the tests showed no diminution in strength; (fresh juice, 4 flies *v; bw*, mean 2.5; 24 hours old juice, 5 flies, mean 2.5). Thus, when freed from enzymes in

this way, the v^+ substance is also stable to oxidation. It was also found that active extracts could be obtained from pupae which had been dried at 100° in the oven.

On evaporating the extract to dryness in vacuo, and extracting with acetone, practically no solids were obtained in the acetone; both tests of this extract taken up in water were negative (see table 1). The residue, on freeing from insoluble matter and taking up in water, was still highly active. The active substance is therefore insoluble in absolute acetone.

TABLE 1

PREPARATION	STRENGTH OF REACTION IN *v*; *bw*	IN *cn bw*
Original juice (2000 pupae in 0.5 cc.)	2.5	1.75
Evaporate; extract with acetone; test the extract	0	0
Take up residue in 0.2 cc. water and filter from bulky precipitate	2.8	2.2
Treat with 0.02 cc. 5% H_2O_2	0.6	0.5
Add charcoal and boil	0.9	0.1

Oxidation of the Substances.—Extracts made in air from pupae first boiled in water or dried at 100°C. are active. The susceptibility to oxidation by air evidently is marked only in the presence of the enzymes of the pupa. Since Dakin[6] has shown that hydrogen peroxide closely imitates many types of biological oxidation, an attempt was made to reproduce the action of the enzymes of the pupa by this reagent. To an active extract, H_2O_2 was added up to a final concentration of 0.2 per cent. The solution was then gently boiled. This treatment greatly weakened the responses to both tests (table 1). No marked difference in the susceptibility of the two substances was indicated, although numerous experiments were carried out under varying conditions with H_2O_2, especially with concentrations $^1/_{10}$ to $^1/_{20}$ of the above. It appears that the activities of both cn^+ and v^+ substances are more or less completely destroyed by peroxide.

Comparison of v^+ *and* cn^+ *Substances.*—In all tests of the aqueous extracts of pupae, the response in the test for cn^+ substance was consistently weaker than that for v^+ substance, by from 0.5 to 1 point. On the other hand, Beadle[5] finds that implantation of Malpighian tubes gives results which are very nearly equal in the two tests. The above experiments give no evidence that cn^+ substance is any more easily destroyed by oxidation than the v^+ substance. It follows either that the cn^+ substance is less easily extractable than the v^+ substance, or that it takes more of the cn^+ substance to produce an equivalent effect. Strong positive results (*cn* 3.0) obtained with hot Ringer extract of Malpighian tubes from only 15 larvae, would suggest that the bulk of the substances are present in these organs, and hence the differences are ascribable to the differences in the ease with which the two substances are extracted. This conclusion is supported by an

experiment with cold Ringer extract of wild type Malpighian tubes; this extract, in *v; bw*, gave a color-value of 2.0, but in *cn bw* only about 0.8.

Finally, proof that the two tests cannot be due to the same substance, previously adduced by transplantation experiments, was given by extracting cinnabar pupae, which should contain only the v^+ substance. About 200 pupae were heated to 100°, crushed and centrifuged in nitrogen as described above, and the clear juice injected; it gave a v^+ test of about 2.5, but the *cn* test was completely negative.

Summary.—1. The substances capable of changing vermilion and cinnabar eye color toward wild type may be obtained in cell-free extracts from wild-type pupae, or the former alone from cinnabar pupae.

2. These substances are heat-stable and water-soluble, and apparently insoluble in acetone or sesame oil. They are, therefore, presumably neither proteins nor enzymes.

3. The cn^+ substance is less readily extracted than is v^+ substance.

4. Both substances are rapidly destroyed by oxidizing enzymes in the pupa juice, or, in the absence of enzymes, by dilute solutions of H_2O_2.

5. A simple method is described for roughly determining the concentration of the substances.

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[6] Dakin, H. D., *Oxidations and Reductions in the Animal Body*, New York (1922).

DEVELOPMENT OF EYE COLORS IN DROSOPHILA: FAT BODIES AND MALPIGHIAN TUBES AS SOURCES OF DIFFUSIBLE SUBSTANCES

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From the work of Ephrussi and Beadle (see Ephrussi and Beadle[1] and Beadle and Ephrussi[2] for summaries) it is known that three diffusible substances are involved in the production of wild type eye color in *Drosophila melanogaster*. This paper is concerned with the normal sources of two of these, v^+ and cn^+ substances. Under certain conditions of genetic constitution these two substances may be produced by eye tissue itself.[1,2] Sturtevant's studies of mosaics of *D. simulans* indicated that v^+ substance

must have another normal source in the fly;[3] and it has been supposed, but not proved, that this was likewise true of cn^+ substance. Tests for the production of v^+ and cn^+ substances by a given organ can be made conveniently by transplanting the organ in question into gentically vermilion (*v*) and cinnabar (*cn*) flies, respectively. Such tests can be made in the late larval stages as shown by positive results from certain eye transplants made at this time.[1,2] In practice, tests for these substances are more sensitive if test animals carrying an eye color "dilution" gene are used. The double recessive apricot vermilion (*w*^{a} *v*) and apricot cinnabar (*w*^{a}; *cn*) have been used extensively. In many of the tests reported here, vermilion brown (*v; bw*) and cinnabar brown (*cn bw*) are used. All of these double recessive eye colors are very light; there is only, at the most, a tinge of color. A positive test is indicated by a modification in the direction of apricot or brown; the maximum change is represented by phenotypes as dark as apricot or brown. In preliminary tests, effects are roughly graded as strong, medium, weak or none: "Strong" represents a complete or nearly complete change. In certain experiments an attempt was made to use a system of numerical grades.

Fat Bodies.—Fat bodies of wild type larvae taken shortly before puparium formation (referred to as "standard" age in all tables) and transplanted to *w*^{a} *v* or *v; bw* larvae of similar age bring about a strong modification of the eye color of the host (table 1), showing that v^+ substance is released from such an implant; evidence to be mentioned later indicates that the fat body actually produces this substance. Unless otherwise specified (table 1), the greater part of one lateral fat body was transplanted, representing perhaps one-fourth to one-third of the total fat body tissue of a larva. There appeared to be no significant differences among the four possible sex-combinations of donor and recipient, and sexes are therefore disregarded in the table. In some experiments an ovary, which is embedded in the fat body, was transplanted with the fat body. This apparently did not influence the result. It will be noted that a few tests are recorded as giving negative results; these presumably represent instances of failure of the fat body to remain in the recipient. Since the fat body normally breaks down during metamorphosis, there is no way of checking this. However, when an ovary was transplanted with the fat body, dissections were made and in all flies in which the implanted ovary was found (indicating that the operation was successful), the results were positive.

In order to determine whether the production of v^+ substance is localized in any one part of the fat body, transplantations of the more or less triangular region of the fat body attached by one margin to the salivary gland (referred to in the table as "head fat") was tested (table 1). There is evidently no marked localization.

Fat bodies from cinnabar larvae produce v^+ substance, as might have

been predicted from eye-transplant experiments.[1,2] Transplants of claret fat bodies also give positive tests although here the amount of v^+ substance produced is apparently a good deal less than that produced by a comparable piece of wild type or cinnabar fat body; again this is in the result expected on the basis of previous studies.

On the assumption that production of v^+ substance by fat bodies might depend on an interaction with other tissues, transplants of wild type fat bodies, with and without ovaries, were made at earlier stages. Transplants made at approximately 21 and 40 hours before puparium formation evidently give results similar to those made at later stages (table 1).

TABLE 1

TESTS FOR THE PRODUCTION OF SUBSTANCES BY TRANSPLANTED FAT BODIES

In Some Experiments Ovaries Were Transplanted Simultaneously; Unless Queried, Success of Ovary Transplants Was Determined by Dissection of Adults

IMPLANT	HOST	HOURS BEFORE PUPARIUM FORMATION	MODIFICATION OF EYES OF HOST				TOTAL
			STRONG	MEDIUM	WEAK	NONE	
+	*w*^{a} *v*	Standard	5	9	1	0	15
+ (with ovary)	*w*^{a} *v*	Standard	3	3	0	0	6
+ (head fat)	*v; bw*	Standard	5	6	2	1	14
cn	*v; bw*	Standard	7	10	1	1	19
ca	*v; bw*	Standard	0	4	4	2	10
+	*w*^{a} *v*	21 ±	4	6	2	0	12
+	*w*^{a} *v*	40 ±	4	1	0	2	7
+ (with ovary)	*w*^{a} *v*	40 ±	4	0	0	0	4
+	*v*	Standard	0	2	0	1	3
+ (with ovary)	*v*	Standard	0	3	0	0	3
+ (with ovary?)	*v*	Standard	0	0	9	0	9
+	*cn bw*	Standard	0	0	0	27	27
+ (with ovary)	*cn bw*	Standard	0	0	0	17	17
+ (head fat)	*cn bw*	Standard	0	0	0	15	15
ca (with ovary?)	*cn bw*	Standard	0	0	0	5	5
+	*cn*	Standard	0	0	0	3	3
+ (with ovary)	*cn*	Standard	0	0	0	7	7

That the portion of fat body transplanted in these tests does not produce sufficient v^+ substance to change vermilion completely to wild type is shown by transplants of wild type fat bodies to vermilion test flies. Table 1 records the modification as "medium," which, as nearly as could be judged, was about half-way from vermilion to wild type.

In striking contrast to the tests of fat bodies for the production of v^+ substance, similar tests for the production of cn^+ substance were invariably negative (table 1). Tests were made of lateral fat bodies with and without an ovary and of "head fat" as defined above. This difference in the test for the two substances considered in conjunction with the tests of extracts reported in the accompanying paper (Thimann and Beadle[4]) constitutes strong evidence for the correctness of the conclusion previously

arrived at on other grounds that v^+ and cn^+ substances are distinct; it becomes increasingly more difficult to argue that v^+ and cn^+ substances actually represent merely different concentrations of a single substance.

Malpighian Tubes.—Transplants of wild type Malpighian tubes to *cn bw* flies at the late larval stage give strong modifications of the color of the eyes of the hosts, in many instances approaching the color of brown flies (table 2). Malpighian tubes obviously release appreciable quantities of cn^+ substance. As in the case of the release of v^+ substance by wild type fat bodies it can be shown that the amount of cn^+ substance released by four tubes (the normal number in *Drosophila*) is not sufficient to change cinna-

TABLE 2

TESTS FOR THE RELEASE OF SUBSTANCES BY MALPIGHIAN TUBES

In Tests Giving Weak Modifications, No Distinction Is Made in the Table between Dorsal and Ventral Tubes. Sexes of Donors and Hosts Are Disregarded

IMPLANT	HOST	MODIFICATION OF EYES OF HOST				TOTAL
		STRONG	MEDIUM	WEAK	NONE	
+; 4 tubes	*cn bw*	11	0	0	0	11
+; 2 ventral	*cn bw*	0	1	0	0	1
+; 4 tubes	*cn*	2	8	2	0	12
+; 2 tubes	*cn*	0	1	0	0	1
+; 1 tube	*cn*	0	0	1	0	1
+; 4 tubes	*v; bw*	19	2	0	0	21
+; 2 tubes	*v; bw*	1	1	0	0	2
cn; 2 dorsal	*v; bw*	0	0	5	1	6
cn; 2 ventral	*v; bw*	0	0	8	0	8
cn bw; 2 dorsal or ventral	*v; bw*	0	0	11	1	12
bri; 2 tubes	*v; bw*	2	0	10	1	13
bri; 2 tubes	*cn bw*	2	6	4	0	12
ca; 2 tubes	*v; bw*	0	0	18	2	20
ca; 2 tubes	*cn bw*	0	0	8	5	13
lt; 2 tubes	*cn bw*	0	0	7	1	8
ma; 2 dorsal	*cn bw*	2	5	3	0	10
ma; 2 ventral	*cn bw*	0	3	4	0	7
w; 2 tubes	*cn bw*	0	0	10	0	10

bar to wild type. The modification of the eyes of *cn* test flies recorded in table 2 as "strong" was such that the eye color appeared to be intermediate between cinnabar and wild type, but somewhat closer to wild type. This result is not surprising when it is remembered that wild type eye tissue itself produces an appreciable amount of cn^+ substance.

Similar tests for v^+ substance show that this substance, too, is released by wild type Malpighian tubes (table 2). Table 2 indicates that v^+ substance is released by *cn* and by *cn bw* Malpighian tubes, but in very definitely smaller amount than that released by wild type tubes. Malpighian tubes of bright (*bri*) larvae release appreciable quantities of both

v^+ and cn^+ substances, but probably less than the quantities released by wild type tubes. Tests of tubes of claret (*ca*), light (*lt*), maroon (*ma*) and white (*w*) larvae show (table 2) that: (1) Claret releases very small amounts of both substances; (2) Light releases a very small amount of cn^+ substance (no test was made for v^+ substance); (3) Maroon releases a moderate amount of both (less than wild type tubes); and (4) white releases a very small amount of cn^+ substance (no v^+ substance test was made).

The above tests of Malpighian tubes show an interesting and probably significant relation between color and release of v^+ and cn^+ or both substances. If we arrange these eye color mutants in a series with wild type according to depth of yellow color of the Malpighian tubes, we find that

$$+ > bw > ma \cong cn > cn\ bw \cong ca \cong lt \cong w,$$

the last four types having approximately colorless tubes. There is apparently a close relation between depth of pigmentation of the Malpighian tubes and the release of the two substances. An understanding of the significance of this apparent relation must await the result of further experiments.

According to table 2, *ca*, *lt* and *w* tubes release small amounts of cn^+ substance. If the test for cn^+ substance in *ca*, *lt* and *w* flies is made by growing *cn* eye implants in these mutants as hosts, *lt* and *w* show no evidence of having less cn^+ substance than has wild type, but *ca* gives a definitely weaker test. Evidently we are dealing here with another instance of localized difference in production of a diffusible substance.[5]

It is possible, as has been implied above, that Malpighian tubes do not produce v^+ and cn^+ substances, but rather accumulate them in the larval stage and release them later. This seems improbable since direct tests of body fluid of late larvae give no indications of the presence of v^+ substance (Ephrussi, Clancy and Beadle[6]). This argument is supported, but not definitely proved, by transplants in which wild type Malpighian tubes taken 24 hours before puparium formation were grown in older *cn bw* test animals (data included in table 3). Apparently these tests are as strong as those with older tubes.

Relation of Amount of Substance to Strength of Test.—From previous work with these diffusible substances it has seemed reasonable to assume that the amount of change produced was a function of the amount of diffusible substance limiting eye pigment development. However, direct tests of this assumption by comparing the implant-host effect of one and two eye implants gave inconclusive results.[7] Tests of the release of cn^+ substance by different numbers of Malpighian tubes are summarized in table 3. Modifications are recorded according to a numerical scheme as described in the accompanying paper (Thimann and Beadle[4]). In table 3 data are

grouped by spacing so that values within groups are strictly comparable; those between groups are less closely comparable. In the first group of tests there is a considerable amount of spread, presumably because ages were not accurately controlled, but it is obvious that two tubes give a stronger effect than one. Dorsal tubes are longer than ventral tubes and appear to give a correspondingly stronger effect. In the second and third groups, where ages were more accurately controlled, the results are more consistent. It should be noted that in the second group the tests of four tubes are not strictly comparable with those of one and two tubes because of an age difference between implant and host. These results support the

TABLE 3

COMPARISONS OF THE MODIFICATIONS BROUGHT ABOUT BY TRANSPLANTING DIFFERENT NUMBERS OF WILD TYPE MALPIGHIAN TUBES TO *cn bw* LARVAE

Sexes of Donors and Hosts Are Disregarded

IMPLANT	HOURS AFTER EGG-LAYING IMPLANT	HOST	MODIFICATION OF EYES OF HOST 5	4	3	2	1	0	MEAN GRADE	TOTAL NUMBER
1 dorsal	Standard	Same			3	3			2.5	6
2 dorsal	Standard	Same	3	3	1	0			4.3	7
1 ventral	Standard	Same	0	0	1	0	3		1.5	4
2 ventral	Standard	Same	3	1	2	0	1		3.7	7
4 tubes	Standard	Same	4	0	2				4.3	6
			MODIFICATION OF EYES OF HOST 4.3	4.0	3.7	3.3	3.0	2.7		
1 dorsal	95–98	Same			1	3	5		3.2	9
2 dorsal	95–98	Same		6					4.0	6
1 ventral	95–98	Same			2	3	3		3.3	8
2 ventral	95–98	Same		4					4.0	4
4 tubes	72–75	95–98	6						4.3	6
1 dorsal	114–117	Same				1	4	1	3.0	6
2 dorsal	114–117	Same	1	2	2				3.9	5
2 dorsal	95–98½	Same	3	1	2				4.1	6
2 ventral	95–98½	Same	1	1	1	2			3.7	5
4 tubes	72–75	96–99½	1			1			3.8	2

assumption that the modification of the eye color of the *cn bw* hosts is some function of the amount of cn^+ substance available.

Extraction of Substances from Malpighian Tubes and Fat Bodies.—As pointed out in the accompanying paper,[4] both v^+ and cn^+ substances can be extracted from wild type larval Malpighian tubes by cold or hot Ringer's solution. This is of interest in connection with the fact, mentioned above, that tests of larval body fluid are negative. Similar attempts to extract these substances from larval fat bodies gave clearly negative results, suggesting that v^+ substance is produced by fat bodies only at a later stage of development.

Tests of Other Organs.—Tests for the production of cn^+ substance by wild type salivary glands (one or two, with and without ovaries), brain tissue, gastric caeca and that portion of the hind gut including the imaginal ring (which regenerates a portion of the hind gut of the imago, Robertson[8]) have all given negative results.

Modification of Ocellus Color.—Vermilion and cinnabar flies have white or very pale ocelli while in wild type and brown flies these organs are brown. It has been noted that *cn bw* flies in which the eye color has been strongly modified by cn^+ substance have unmodified ocelli while *v; bw* flies showing a strong modification of eye color also show a strong modification of ocellus color toward brown. This suggests that ocellus color in cinnabar is autonomous in development, but that in vermilion it is not. However, it remains possible that higher concentrations of cn^+ substance will modify the ocelli of cinnabar flies.

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THE REGENERATION OF PLATE ROWS IN MNEMIOPSIS LEIDYI, AGASSIZ

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The power of Mnemiopsis to regenerate parts of its body that have been injuried in nature or that have been removed during experimentation is remarkable (Mortensen, 1913, and Coonfield, 1936 *a*). The rapidity of regeneration, the exactness reached in regulating the reformed organs, and the transparency of its body makes this animal an excellent one for experimentation. Organs such as plate rows and the apical organ can be observed clearly during their entire period of regeneration. The unusually rapid rate at which these organs reform is interpreted by me as being due either to a rapid movement and realignment of cells from the remaining parts of the removed organs or to the migration of nonspecialized cells into the wound area. This interpretation can be tested in this animal by

removing a part of an organ, leaving thus some of its cells in the animal, and by removing this organ entirely. Therefore the purpose of experiments cited here was to test this interpretation by cutting out a part of a plate row and by removing the whole of a plate row. Histological evidence will not be given since a successful technique for this has not been developed.

Experiments.—The method of keeping and observing the experimental animals that are referred to here was the same as for those reported by Coonfield (1936 *a*). Three types of experiments were performed. In one experiment, a small section of a plate row was removed from the mid-section of the row (Fig. 5), or from near the apical end of the row, or from near the oral end of the row. In the second type of experiment the entire row on which plates normally are found was cut out (Fig. 4). In this experiment a small part of the row canal was left in place both at the oral end and at the apical end of the animal. These parts of the row, however, lie in the mesogleal layer and have no plates above them. In the third type of experiment the two ends of the animal were cut off (Fig. 2) so as to remove the row and canal connections at the infundibulum and those connections at the bases of the auricles. A part of a row or the entire row (Fig. 3) was then removed from the remaining mid-piece of the animal.

In order to understand what is included in the term plate row the following description is given. There are eight plate rows, four adtentacular and four adesophageal, in the body of Mnemiopsis. Each row connects to the infundibulum as a canal at the apical end of the animal. Each passes from within the mesoglea in this region to near the surface of the body. At the oral end of the animal each of the four adtentacular rows passes into an auricle while each of the four adesophageal rows sinks into the mesoglea as a canal. Plates are found on the rows only in the regions where the canals are near the surface of the animal's body. A plate row therefore consists of a canal which is without plates at both ends of the body and which has plates on it between these two ends. The canal sends out a lateral branch on each side to the base of each plate.

Short Section of a Row Removed.—A wedge-shaped piece of a row containing 10–20 plates was cut out of the animal. Such a piece was removed from the mid-part of a row and also from near either end of a row. The ends of the row were observed during the entire period of regeneration, since each had been stained at the time of cutting by Nile blue sulphate in agar blocks. The operations were done on both adtentacular and adesophageal rows with no apparent difference in the results.

Immediately following the removal of a wedge-shaped piece of a row the lateral edges of the wound moved together and fused. Within three hours after the operation a considerable number of cells migrated from within the mesoglea and concentrated at the healed region between the remaining ends of the row. These cells formed a line (cord) in this region and as this

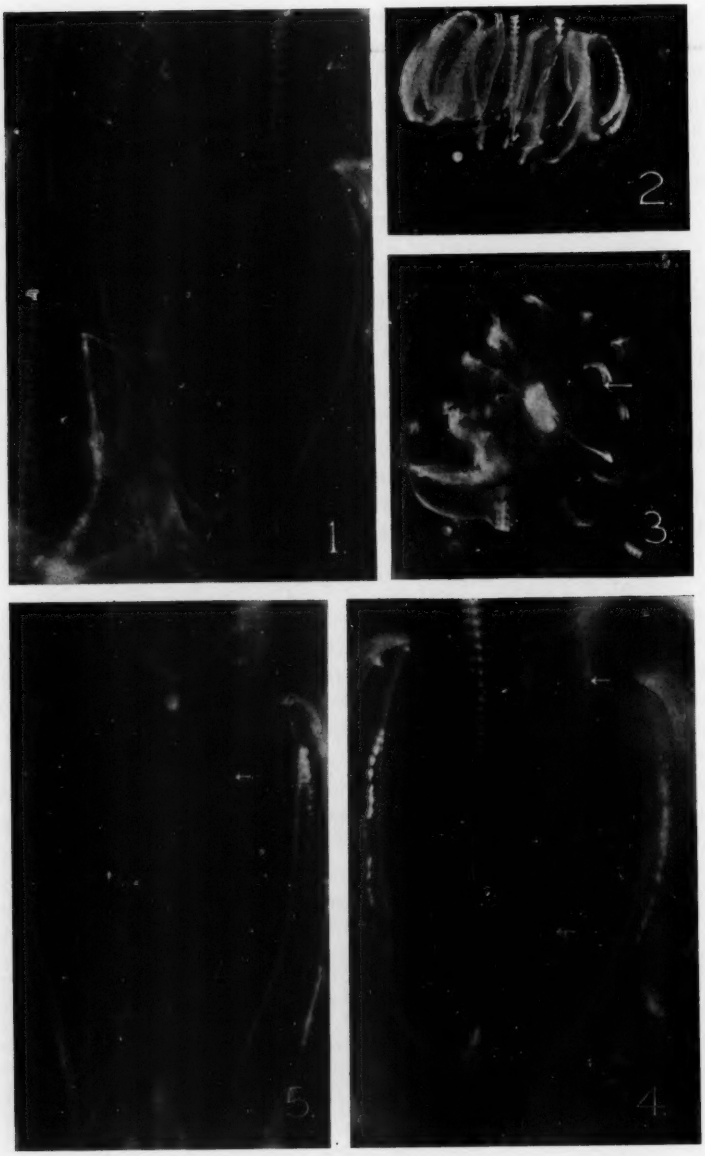

PLATE I

(Description on opposite page.)

was occurring the muscle strands anchored both at the cut ends of the row and deep in the mesoglea beneath contracted to bring the ends of the row gradually together until they fused (Fig. 5). Within an hour after the ends of the row had been brought together, the cellular mass in this region hollowed out to make the canal continuous. Since the ends of the row were stretched as they were pulled together by muscles, the plates of the row were separated from each other. Within about three days after the canal became continuous new plates began to form on the row in between the old plates.

Entire Row Removed.—The entire length of a row on which plates regularly grow was removed. By this operation the two ends of the canal were left in place. Thus some of the endodermal part at each end of the row was left in place after the operation. The wound healed soon after the operation and within three hours cells migrating from the mesoglea concentrated at the healed region and formed a cord between the remaining ends of the canal. The ends of the canal were pulled toward each other to some extent, due to the contracting muscle strands of the row. These ends, however, did not move entirely across the wound area (Fig. 4). The cord which had been formed by the mesogleal cells hollowed out and became a canal connecting the two ends of the old canal. Food was seen to pass through this canal, proving thus that it was continuous. Three days after this time plates began to form above this canal.

Row Removed from Mid-Piece.—The mid-piece of the body of Mnemiopsis was obtained by cutting the animal across just below its infundibulum and above its auricles (Fig. 2). Thus all canal connections were parted by two cuts. By cutting out the entire remaining portion of a plate row from this mid-piece the endoderm in this particular region was removed. Either a portion of a row or an entire row was removed from the mid-piece. These operations were done on both the adtentacular and the adesophageal rows.

DESCRIPTION OF PLATE I

Fig. 1. Part of the adtentacular surface of an injured specimen collected in its natural habitat. The two rows at the left of the photograph show regenerating plates.

Fig. 2. The mid-piece of a specimen immediately after its apical and oral ends have been removed.

Fig. 3. Photograph of the apical end of a mid-piece healing at its cut surface. The arrow indicates the region from which an adtentacular row had been removed completely. Photographed 24 hours after the operation.

Fig. 4. A regenerating adtentacular row following its complete removal is shown on the right of this photograph. The new canal can be seen and it occupies the region between the two arrows. Mid-way between these two arrows a mass of materials can be seen. This mass of materials was produced by the migrating cells within the mesoglea. Photographed 12 hours after the operation.

Fig. 5. Photograph of the pulling together of the two cut regions of the remaining ends of an adtentacular row following the removal of its mid-part. The fusing cut regions are indicated at the arrow. Photographed 12 hours after the operation.

The wound made by the removal of a row healed quickly and soon afterward the healing at the cut ends of this mid-piece was complete (Fig. 3). When a portion of a row was left in place a new row regenerated from this remaining part. There was considerable stretching of the part of the old row and at the same time many of the cells within the mesoglea concentrated at the wound. These cells probably contributed to the formation of the new canal and row. The new row was complete except for its plates within three days. Following the removal of the entire row, new row canals which originated at the two ends of the mid-piece were complete at the end of four days. New plates began to form on the regenerated canal one day after the canal was formed, following the removal of a portion of the row and also following the removal of an entire row. The time and procedure of regeneration of part and whole rows were the same in the adtentacular and adesophageal regions.

In order to ascertain whether the line (cord) formed by the migrating cells of the mesoglea which followed the removal of a part or the whole of a row was only a part of the mechanism of ordinary wound healing, the following experiment was executed. A wedge-shaped piece of the body involving both ectoderm and mesoglea was removed from between the plate rows. This was done between the adtentacular and the adesophageal rows. The wounds healed promptly and without any line (cord) formation and also without any visible migration of the mesogleal cells.

Discussion.—Records of experiments cited in this report and evidence brought out previously (Coonfield, 1936 *a*) show that regeneration in Mnemiopsis is quite rapid. I have described experiments which were designed to test the hypothesis that rate of regeneration as well as the process itself in Mnemiopsis depends either on migrating cells from the remains of extirpated organs or on migrating nonspecialized cells. It is quite possible that regeneration in Mnemiopsis is due to both of these processes.

In the experiments wherein parts of a plate row were left in place, I am certain that cells migrated from these parts into the wound gap and contributed to the regeneration of the removed part. This conclusion is based on results of the experiments where a portion of a row was removed from the whole animal and from an isolated mid-piece. The cut ends of the row were stained with Nile blue sulphate to enable me to observe any change in their location. These ends moved toward each other until fusion occurred. During the migration the ends of the old row were pulled by the mucle strands. This resulted in a thinning out of the stretched canal. While this was taking place a great many cells within the mesoglea concentrated to form a definite line between the ends of the old row. Whether these cells actually contributed to the new canal I cannot be certain. In the experiment where a portion of a row was cut out of a mid-piece of the animal, the new canal formed from the remaining end of the row. Here too

a considerable stretching of the remaining end of the row occurred. Regeneration actually began at this end and continued along the region occupied previously by the removed section of the row. In the experiments in which the whole row was removed from a mid-piece of the animal, regeneration of this row began at each end of this piece. Since the wound at the two cut surfaces of the mid-piece healed within three hours, and since the ends of the other seven canals were brought together before the rows started to regenerate, I assume that endodermal cells from these canals contributed to the formation of the new canal and row. Here again cells within the mesoglea were seen to move and then concentrate to form a line in the area from which the row had been cut. The results therefore show that cells from the remaining parts of an extirpated plate row and cells in the mesoglea were active in the regeneration of the lost parts of the row.

The experiments, in which an entire row on which plates usually grow was extirpated, left a small amount of the canal in place in the mesoglea. These remaining parts of the canal stretched to some extent but failed to cover the operated region completely (Fig. 4). Cells from the mesoglea were seen to migrate into the wound area and to form a definite line between the two ends of the canal. This line of concentrated cells hollowed out to form a canal continuous from end to end. The lining up of these cells in the exact region occupied previously by the removed section of the row contributes further to the evidences of polarity within Mnemiopsis (Coonfield, 1936 *b*). The evidence based on the results of these experiments shows that the nonspecialized cells in the mesoglea migrate to the wound area following the removal of a row and form a new canal. Later plates were formed along and above this new canal to complete the regeneration of the extirpated plate row. It is interesting to note further that as the plates formed along this new canal and also along the regenerated canal following the removal of parts or the whole of a row, as described in the other experiments in this report, these plates regenerated first at the apical end of the row.

In conclusion the experiments discussed here show that when a portion of a plate row was removed from a whole Mnemiopsis, or when a portion of a row or the whole of a row was removed from a mid-piece of this animal, regeneration is due primarily to the migrating endodermal cells from the remaining parts of the canal or from neighboring canals. These experiments show further that when the entire canal above which plates grow was removed from the whole body of Mnemiopsis, regeneration is due primarily to the migrating nonspecialized cells from the mesoglea. Therefore these two processes account in part for the rate of regeneration as well as for the mechanism itself in this animal.

Conclusions.—1. A plate row regenerates following its removal in part or as a whole.

2. The sequence in the regeneration of a plate row is as follows: healing, stretching of the remaining part or parts of the canal and concentration of mesogleal cells at the wound area, fusion of parts of the canal, hollowing out at the region of fusion and formation of plates above the new canal.

3. Cells from the remaining part or parts of a row or from neighboring rows and also nonspecialized cells in the mesoglea take an active part in the regeneration of plate rows.

4. Contracting muscle strands which are anchored to the plate row and also in the mesoglea aid in the reformation of a row following its removal in part or as a whole.

* Contribution No. 18 from the Department of Biology, Brooklyn College.

B. R. Coonfield, "Regeneration in Mnemiopsis leidyi, Agassiz," *Biol. Bull.*, **71**, 421 (1936 *a*).

B. R. Coonfield, "Apical Dominance and Polarity in Mnemiopsis leidyi, Agassiz," *Biol. Bull.*, **70**, 460 (1936 *b*).

Th. Mortensen, "On Regeneration in Ctenophores," *Vidensk. Meddel. fra Dansk naturh. Foren.*, **66**, 45 (1913).

IMMERSION OF THE FOURIER TRANSFORM IN A CONTINUOUS GROUP OF FUNCTIONAL TRANSFORMATIONS

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The Fourier transform $g(u)$ of a function $f(x)$ is defined by

$$g(u) = \frac{1}{\sqrt{2\pi}} \int f(x) e^{iux}\, dx \qquad (1)$$

where $\int$ means integration from $-\infty$ to $+\infty$. This possesses* the inversion formula

$$f(x) = \frac{1}{\sqrt{2\pi}} \int g(u)\, e^{-iux}\, dx. \qquad (2)$$

Evidently if we write F for the operation performed on f in (1) then (1) and (2) can be written

$$g(u) = F f(x), \quad f(-x) = Fg(u) = F^2 f(x),$$

from which it follows that

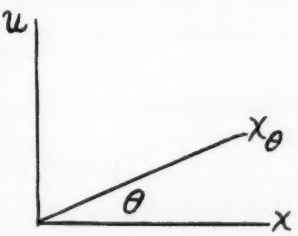
$$f(x) = F^4 f(x) \qquad (3)$$

so the transformation is of period 4. Another way of regarding (2) is that the operation there performed on $g(u)$ is F^{-1} for this is consistent with

$$f(x) = F^{-1}\,g(u) = F^{-1}\,Ff(x). \tag{4}$$

Hence the operation F generates a cyclic group of order 4 which is isomorphic with the group of rotations of a plane about a fixed point through multiples of a right angle. Now every continuous group of transformations is generated by an Hermitian operator, and conversely every Hermitian operator generates a group of unitary transformations.† Hence there exists a continuous group of functional transformations containing the ordinary Fourier transforms as a subgroup. In this paper the continuous group is explicitly found. It will not, however, be necessary to make further reference in the work that follows to the general immersion theory.

It is convenient to introduce a group space as shown in the figure in which the notation x_θ is assigned to the argument of a function which is generated out of $f(x)$ by application to it of the functional transformation F_θ. In this notation $F_\theta f(x)$ will be a transformed function of the argument x_θ which we may write as $f_\theta(x_\theta)$. Evidently x is x_0 and $f(x)$ is more explicitly $f_0(x_0)$ in this notation.



Likewise the ordinary Fourier transform becomes $F_{\pi/2}$ and we have $u = x_{\pi/2}$ and

$$g(u) = f_{\pi/2}(x_{\pi/2}).$$

The kernel of the integral which represents the operation F will depend on two variables: one is x_α where the operation is applied to a function $f_\alpha(x_\alpha)$; the other is $x_{\alpha+\theta}$ corresponding to the fact that the operation "rotates" the function through an angle θ in the group space. The general integral representation of (1) becomes in this notation

$$f_{\alpha+\theta}(x_{\alpha+\theta}) = \int K_\theta(x_\alpha, x_{\alpha+\theta})\, f_\alpha(x_\alpha)\, dx_\alpha. \tag{5}$$

The problem is that of finding the kernel which represents the operation F_θ. Before proceeding to this question we may observe that the kernel will be necessarily singular for $\theta = 0$ and $\theta = \pi$. For $\theta = 0$ the operation should reduce to the identical transformation

$$f_\alpha(x') = \int K_0(x_\alpha, x'_\alpha)\, f_\alpha(x_\alpha)\, dx_\alpha \tag{6}$$

which can only be forced into this form by the introduction of an improper function $\delta(x)$ defined by the properties

$$\int \delta(x)dx = 1 \text{ and } \delta(x) = 0 \text{ for } x \neq 0. \tag{7}$$

In terms of $\delta(x)$ we have

$$K_0(x_\alpha, x'_\alpha) = \delta(x_\alpha - x'_\alpha). \tag{8}$$

Similarly for $\theta = \pi$ we must have, since $x_{\alpha+\pi} = -x_\alpha$

$$f_{\alpha+\pi}(x_{\alpha+\pi}) = f_\alpha(-x_\alpha)$$

so the kernel in this case also becomes the improper $\delta(x)$

$$K_\pi(x_\alpha, x_{\alpha+\pi}) = \delta(x_\alpha + x_{\alpha+\pi}). \tag{9}$$

Otherwise one has no reason to expect singular behavior of the kernel. In particular from the ordinary theory of Fourier transforms,

$$\begin{aligned} K_{\pi/2}(x_\alpha, x_{\alpha+\pi/2}) &= \frac{1}{\sqrt{2\pi}} \exp\{i\, x_\alpha\, x_{\alpha+\pi/2}\} \\ K_{3\pi/2}(x_\alpha, x_{\alpha+3\pi/2}) &= \frac{1}{\sqrt{2\pi}} \exp\{-i\, x_\alpha\, x_{\alpha+3\pi/2}\}. \end{aligned} \tag{10}$$

An important property of the Fourier transform which it is desirable to preserve is

$$\int |g(u)|^2\, du = \int |f(x)|^2 dx$$

which in our notation becomes

$$\int |f_\theta(x_\theta)|^2 dx_\theta = \int |f(x)|^2\, dx \tag{11}$$

for all θ. Proceeding formally we have

$$\int |f_\theta(x_\theta)|^2 dx_\theta = \int\int\int f(x)\, K_\theta(x, x_\theta)\, \overline{f(x')}\, \overline{K_\theta(x', x_\theta)}\, dx\, dx'\, dx_\theta$$

which will be true if

$$\int K_\theta(x, x_\theta)\, \overline{K_\theta(x', x_\theta)}\, dx_\theta = \delta\,(x - x'). \tag{12}$$

The law of combination of transformations is given by:

$$\begin{aligned} f_{\theta+\varphi}(x_{\theta+\varphi}) &= \int f_\theta(x_\theta)\, K_\varphi(x_\theta, x_{\theta+\varphi})\, dx_\theta \\ &= \int\int f(x)\, K_\theta(x, x_\theta)\, K_\varphi(x_\theta, x_{\theta+\varphi})\, dx\, dx_\theta \\ &= \int f(x)\, K_{\theta+\varphi}(x, x_{\theta+\varphi})\, dx. \end{aligned}$$

The last two expressions are equivalent if

$$K_{\theta+\varphi}(x, x_{\theta+\varphi}) = \int K_\theta(x, x_\theta) K_\varphi(x_\theta, x_{\theta+\varphi})\, dx_\theta. \tag{13}$$

The transformation with parameter $-\theta$ must be reciprocal to that with parameter θ so

$$K_{\theta-\theta}(x, x') = \delta(x - x') = \int K_\theta(x, x_\theta)\, K_{-\theta}(x_\theta, x')\, dx_\theta.$$

This will be consistent with (12) if we require of the kernels that

$$K_{-\theta}(x_\theta, x') = \overline{K_\theta(x', x_\theta)}. \tag{14}$$

We may find the desired kernel as follows. With each variable x_θ we associate a Hermitian operator X_θ. In particular write $X_0 = X$ and $X_{\pi/2} = U$ and demand that the operators X and U obey the commutation law

$$UX - XU = -i. \tag{15}$$

For application to functions for x the operators X and U can be explicitly represented by

$$Xf(x) = xf(x) \qquad Uf(x) = -if'(x). \tag{16}$$

We then define the operator for X_θ in this same representation as being the same combination of X and U as holds for x_θ in terms of x and u regarded as ordinary coördinates in the group space,

$$X_\theta = sU + cX \tag{17}$$

where $s = \sin\theta$ and $c = \cos\theta$.

The proper functions $P(x, x')$ of the operator X satisfy

$$XP(x, x') = x'P(x, x') \text{ or } (x - x')P(x, x') = 0.$$

Hence $P(x, x') = 0$ for $x \neq x'$ and so with a suitable normalization $P(x, x')$ corresponds to the kernel for the identical transformation. Similarly the proper function for U, say $Q(x, u)$ satisfies

$$-i\frac{\partial}{\partial x}Q(x, u) = uQ(x, u)$$

which gives $Q(x, u) = e^{iux}$ which if properly normalized gives the kernel of the ordinary Fourier transform. These facts suggest that the proper functions of X_θ in this representation will give the general kernel $K_\theta(x, x_\theta)$ after a suitable normalization. These proper functions satisfy

$$\left(-is\frac{\partial}{\partial x} + cx\right)K_\theta(x, x_\theta) = x_\theta K_\theta(x, x_\theta)$$

which gives

$$K_\theta(x, x_\theta) = \exp\left\{-\frac{ic}{2s}x^2 + \frac{ixx_\theta}{s}\right\}$$

to within a factor which is an arbitrary function of x_θ. Since $c(\theta) = c(-\theta)$ and $s(\theta) = -s(-\theta)$, equation (14) determines the dependence on x_θ so we have except for a normalizing constant C,

$$K_\theta(x, x_\theta) = C \exp\left\{ -\frac{ic}{2s} x^2 + \frac{ixx_\theta}{s} - \frac{ic}{2s} x_\theta^2 \right\}.$$

The magnitude of C has to be determined by (12) which yields the equation

$$|C|^2 \exp\left\{ -\frac{ic}{2s} (x^2 - x'^2) \right\} \int \exp\left\{ \frac{i(x-x')x_\theta}{s} \right\} dx_\theta = \delta(x-x').$$

The integral here is divergent but is the same one which is interpreted as having the value $2\pi s\, \delta(x-x')$ when this formalism is applied to the ordinary Fourier transform. Hence in a formal sense the kernel has the property (12) provided that

$$|C| = (2\pi s)^{-1/2}.$$

Finally we have to verify that the kernel has the group property expressed in (13). In doing this we make the restriction that neither θ nor φ nor $\varphi + \theta$ is equal to 0, $\pi/2$, π or $3\pi/2$. Writing $C = (2\pi s)^{-1/2} e^{i\delta}$ and c' and s' for $\cos\varphi$ and $\sin\varphi$, we have to compute

$$\frac{e^{i2\delta}}{2\pi\sqrt{ss'}} \int \exp\left\{ -\frac{ic}{2s} x^2 + \frac{ixx_\theta}{s} - \frac{ic}{2s} x_\theta^2 - \frac{ic'}{2s'} x_\theta^2 + \frac{ix_\theta x_{\theta+\varphi}}{s'} - \frac{ic'}{2s'} x_{\theta+\varphi}^2 \right\} dx_\theta.$$

The integral may be computed by elementary methods giving the result

$$\frac{e^{i(2\delta \mp \pi/4)}}{\sqrt{2\pi s''}} \exp\left\{ -\frac{ic''}{2s''} x^2 + \frac{ixx_{\theta+\varphi}}{s''} - \frac{ic''}{2s''} x_{\theta+\varphi}^2 \right\}$$

where the ambiguous sign is $-$ if $s''/ss' > 0$ and $+$ if $s''/ss' < 0$ and c'' and s'' are written for $\cos(\theta + \varphi)$ and $\sin(\theta + \varphi)$. This will be exactly equal to $K_{\theta+\varphi}(x, x_{\theta+\varphi})$ if we choose $\delta = \pi/4$ for angles in the first two quadrants and $\delta = 3\pi/4$ for angles in the third and fourth quadrants. Hence the kernel is completely determined for angles other than 0 or π to be

$$K_\theta(x,x_\theta) = \frac{e^{i\delta}}{\sqrt{2\pi s}} \exp\left\{ -\frac{ic}{2s} x^2 + \frac{ixx_\theta}{s} - \frac{ic}{2s} x_\theta^2 \right\}. \qquad (18)$$

For $\theta = 0$ we define the functional transformation to be the identity. For $\theta = \pi$ we define it as

$$f_{\alpha+\pi}(x_{\alpha+\pi}) = e^{i\pi/2} f_\alpha(-x_{\alpha+\pi}),$$

the factor $e^{i\pi/2}$ being introduced for a reason which appears in the next paragraph.

We observe that with the values of δ as determined in the last paragraph the transformation for $\theta = \pi/2$ differs from the ordinary Fourier transform only because of the inclusion of the factor $e^{i\pi/4}$. This determination of the phase has the property both for the ordinary Fourier transform and for our generalization that if a succession of transforms be made such that their parameter values, $\theta_1, \theta_2, \theta_3 \ldots$. add up to 2π, we do not get the identity but get instead the original function multiplied by -1. The representation of the group that has been found is thus a double-valued one and does not reproduce the identity until the sum of the parameter values of the successive transforms is equal to 4π. It is evident that the factor introduced in the special definition of the transform for $\theta = \pi$ is just what is needed to be in accord with these properties.

It will be of interest also to examine the behavior of $f_\theta(x_\theta)$ as $\theta \longrightarrow 0$. For $\theta = 0$ the transformation gives the identity, but for θ small $f_\theta(x_\theta)$ is rather different from $f(x)$, as may be seen from a qualitative inspection of the properties of the kernel (18). Writing $i\Phi$ for the argument of the exponential function in (18) we see that for θ small the coefficients of x^2, xx_θ and x_θ^2, will be large. Hence the kernel will be a rapidly oscillating function of x except in the neighborhood of $x = x_\theta/c$ where $\dfrac{\partial\Phi}{\partial x}$ vanishes.

Hence if $f(x)$ is a smooth function the chief contributions to $f_\theta(x_\theta)$ will come from the values of $f(x)$ for x near to x_θ/c. To bring out this fact we may write Φ in the form

$$\Phi = -\frac{c}{2s}(x - x_\theta/c)^2 + \left(\frac{1}{sc} - \frac{c}{2s}\right)x_\theta^2.$$

The second term is independent of x and therefore $f_\theta(x_\theta)$ will contain a factor $\exp\left\{i\left(\dfrac{1}{sc} - \dfrac{c}{2s}\right)x_\theta^2\right\}$. The first term has rapid oscillations except near $x = x_\theta/c$. The zeros of $\cos\dfrac{c}{2s}(x - x_\theta/c)^2$ that are nearest to x_θ/c occur for $x = x_\theta/c \pm \sqrt{\pi s/c}$ and beyond these the kernel oscillates rapidly. Hence for θ small and $f(x)$ smooth the integral of $f(x)$ occurring in the transform will be very roughly of the order of

$$Av[f(x)].\exp\left\{i\left(\frac{1}{sc} - \frac{c}{2s}\right)x_\theta^2\right\}$$

where $Av[f(x)]$ is an average of $f(x)$ over a range of the order $x_\theta/c - \sqrt{\pi s/c}$ to $x_\theta/c + \sqrt{\pi s/c}$. As $\theta \longrightarrow 0$ this approaches the value $f(x_\theta)$ so $f_\theta(x_\theta)$ becomes qualitatively equal to

$$e^{i(1/sc - c/2s)x_\theta^2} f(x_\theta).$$

The exponential factor, however, becomes a highly singular function of x_θ as $\theta \longrightarrow 0$ so the functional transform does not continuously approach the identity. However, it does have the property that

$$|f_\theta(x_\theta)| \longrightarrow |f(x)|$$

as $\theta \longrightarrow 0$ for smooth functions.

It is a pleasure to acknowledge my indebtedness to helpful conversations with several of my colleagues, especially Professors J. von Neumann, H. Bohnenblust and S. Bochner.

* See, for example, N. Wiener, *The Fourier Integral*, Cambridge University Press, p. 69 (1933).

† Stone, M. H., *Proc. Nat. Acad. Sci.*, **16**, 173 (1930); *Annals of Math.*, **33**, 643 (1932).

LATTICES AND BICOMPACT SPACES

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In this note there are announced certain new results concerning the interrelations between distributive lattices and topological spaces.

THEOREM 1. *Given a distributive lattice* L *with zero and unit, there is a bicompact* T_1*-space* S, *a basis for whose closed sets is a lattice-homomorphic image of* L.

COROLLARY. *This basis for the closed sets of* S *is isomorphic to* L *if and only if* L *has the property that if* a *and* b *are different elements of* L *there exists an element* c *of* L *such that one of* ac *and* bc *is zero and the other is not zero.*

THEOREM 2. *If* R *is a* T_1*-space then the bicompact* T_1*-space* S *obtained by applying the process of Theorem 1 to the lattice of the closed sets of* R *is such that* S *contains a dense subset* R′ *homeomorphic to* R; *if* f *and* g *are closed disjoint subsets of* R, F *and* G *their correspondents in* S *under the homeomorphism* $R \rightleftarrows R'$, $\overline{F}$ *and* $\overline{G}$ *the closures in* S *of* F *and* G, *then* $\overline{F}$ *and* $\overline{G}$ *are also disjoint; in the sense of Čech, the homology theory of* R *is identical with that of* S *and dimension* R = *dimension* S.

SPECIAL CASE.[1] S *is a Hausdorff space* (and hence normal) *if and only if* R *is a normal space.*[2]

The method is as follows. Let L be a distributive lattice with zero and unit. A collection C of elements of L with the property that the intersection of any finite number of elements of the collection is not zero, while C is a proper sub-collection of no collection having the same property, will

be called a *point*,[3] and the elements of the collection will be called the *coördinates* of the point. S will denote the set of all points.

To each element a of L we associate a set of points of S, called the *basic* a-*set*, consisting of all points which have a as one of their coördinates. It is then shown that if a and b are any elements of L the intersection of the basic a-set and the basic b-set is the basic ab-set, and the sum of the basic a-set and the basic b-set is the basic $a + b$-set. It is also shown that different elements of L determine different basic sets if and only if L has the property stated in the corollary to Theorem 1.

The set S is now turned into a space by defining a *closed* set as the intersection (of a finite or infinite number) of basic sets. It is shown that S is then a bicompact T_1-space, and this proves Theorem 1 and its corollary.

Now let L be the lattice of the closed sets of a T_1-space R. It is easily seen that the collection of all closed sets of R containing a given point p of R (p itself is one such closed set) is a point of S; such a point of S is called an *ordinary* point. Conversely let P be a point of S. If the closed sets of R which are the coördinates of P have a point p of R in common (they surely have not more than one point in common), then p itself is one of the coördinates of P, so that P consists of the collection of all closed sets of R containing p; hence P is an ordinary point. But if the closed sets of R which are the coördinates of P have no point of R in common, P is called an *ideal* point.

The R' of Theorem 2 is then taken to be the set of all ordinary points of S.

[1] The sufficiency part of the special case has been given (in unpublished proofs) by Professors Čech and Lefschetz, by use of the method of "projection spectra" or "developments." The present very simple method has, in addition to somewhat greater generality, interest in itself, in that it entirely avoids use of projections.

In Professor M. H. Stone's recent exhaustive work on *Boolean Rings and General Topology* (forthcoming in vol. 41 or 42 (1937) of the Trans. Am. Math. Soc.) the same sort of general method has been applied. But in that work the approach is largely the algebraic one which arises from the problem of representations of Boolean rings, and this has necessitated a detailed algebraic analysis. Here the point of view is the more geometrical one that the topology of a space is completely determined by the lattice of its closed sets; hence it is possible to proceed very quickly to a statement such as that about the homology theory.

[2] In terms of the structure of the lattice L of the closed sets of R we could require: if f and g are elements of L and $fg = 0$ then there exist f' and g' in L such that $f' + g' = 1$, $ff' = gg' = 0$. This is of course the formulation of normality in terms of closed sets only.

[3] A point of S is shown to be a divisorless additive ideal of L which does not contain zero, and conversely.

NOTE ON THE CURVATURE OF ORTHOGONAL TRAJECTORIES OF LEVEL CURVES OF GREEN'S FUNCTION

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Although the shape of the level curves of Green's function for a plane region has been widely studied,[1] the shape of their orthogonal trajectories seems to have been somewhat neglected. That is to say, although the images of concentric circles (Kreisbilder) in a smooth conformal map have been studied, the images of all lines through their common center (Radienbilder) have been much less studied. It is the object of the present note to devote some attention to the curvature of the latter.

We take as point of departure the following theorem:

THEOREM 1. *Let* R_1 *be a region of the extended* (x, y)*-plane containing the point at infinity in its interior; the boundary* B_1 *of* R_1 *is limited. Let there exist Green's function* $G(x, y)$ *for* R_1 *with pole at infinity. Let* $\{T_1\}$ *denote the set of orthogonal trajectories to the level curves* C_r: $G(x, y) = \log r$.

If R_1 *is simply connected, the tangent to an arbitrary curve* T_1 *at an arbitrary point of* T_1 *must cut* B_1. *The set of asymptotes to all curves* T_1 *is identical with the set of lines through a single point* D_1, *the conformal center of gravity of* R_1. *Every straight line through* D_1 *cuts* B_1.

If R_1 *is multiply connected, the tangent to an arbitrary curve* T_1 *at an arbitrary point of* T_1 *must either cut* B_1 *or separate two components of* B_1. *The set of asymptotes to all curves* T_1 *is identical with the set of lines through a single point* D_1, *the conformal center of gravity of* R_1. *Every such straight line through* D_1 *either cuts* B_1 *or separates two components of* B_1.

The curves T_1 are the lines of force corresponding to a distribution of positive matter on B_1, provided B_1 is sufficiently smooth. Under these circumstances, it is therefore intuitively obvious that the tangent to an arbitrary curve T_1 at an arbitrary point must cut B_1 if R_1 is simply connected and must either cut B_1 or separate two components of B_1 if R_1 is multiply connected. A formal proof of the corresponding fact, whether B_1 is smooth or not, is not difficult.[2]

Let us fasten our attention on a particular curve T_1, and study the tangent to T_1 at a point P of T_1, where P is allowed to become infinite. The tangent to T_1 at P always passes through the center of curvature at P of the curve C_r on which P lies. When P becomes infinite, this tangent approaches the asymptote to T_1, and the center of curvature of C_r at P approaches D_1.[3] Thus the asymptote to T_1 passes through D_1. There exists a curve T_1 whose asymptote has any given direction, by interpretation of curves T_1 as the images of radii in the smooth conformal map of a

circle, so the set of asymptotes to all curves T_1 is identical with the totality of lines through D_1.

The point D_1 seems first to have been introduced by Lucas,[4] namely in the case that B_1 is defined by an equation $|p(z)| = \text{const.}$, where $p(z)$ is a polynomial in z. Here the point D_1 is simply the center of gravity of the roots of $p(z)$, and Lucas shows that the asymptotes of the curves $arg[p(z)] = \text{const.}$ pass through D_1. In the case that R_1 is simply connected, the point D_1 was studied by Frank and Löwner,[5] but not in connection with the properties expressed in Theorem 1. Frank and Löwner showed that D_1 is the common center of gravity of all the curves C_r with suitably distributed positive spreads, so the name "conformal center of gravity" is appropriate for D_1. This relation of D_1 to the center of gravity of curves C_r was extended by Walsh (loc. cit., §14) to multiply connected regions R_1. The fact that the asymptote to every curve T_1 either cuts B_1 or separates two components of B_1 can be proved both from the corresponding property for tangents to the curves T_1 and from the interpretation of D_1 as the center of gravity of the loci C_r.

By either an inversion in a circle or by a reciprocal transformation of the complex variable, Theorem 1 yields the following theorem, in which the term "circle" is used to include also straight lines.

THEOREM 2. *Let* R *be a region of the extended* (x,y)*-plane, with boundary* B. *Let there exist Green's function* G(x, y) *for* R *with pole in the finite point* O. *Let* {T} *denote the set of orthogonal trajectories to the level curves* C_r: $G(x, y) = \log r$.

If R *is simply connected, the circle through* O *tangent to an arbitrary curve* T *at an arbitrary point of* T *must cut* B. *As a limiting case, every circle osculating a curve* T *at* O *must cut* B. *The totality of such osculating circles for all curves* T *is precisely the set of circles through* O *and through another fixed point* D *depending on* O. *There exists no circle that separates both* O *and* D *from* B.

If R *is multiply connected, the circle through* O *tangent to an arbitrary curve* T *at an arbitrary point of* T *must either cut* B *or separate two components of* B. *As a limiting case, every circle osculating a curve* T *at* O *must either cut* B *or separate two components of* B. *The totality of such osculating circles for all curves* T *is precisely the set of circles through* O *and through another fixed point* D *depending on* O. *There exists no circle that separates both* O *and* D *from* B.

In Theorem 2 the point at infinity may be an interior, exterior, or boundary point of R.

Theorem 2 is concerned with the orthogonal trajectories T for a specific Green's function with assigned pole O. If R is simply connected, the set of all such curves T for all choices of O is precisely the set of images in R

(under the smooth conformal mapping of R onto the interior of the unit circle T) of the circles orthogonal to T.

There are certain special geometric situations under Theorems 1 and 2 that deserve explicit mention.

If the region R_1 has a center of symmetry, the point D_1 coincides with that center of symmetry. If O is a center of symmetry for R, the corresponding point D lies at infinity.

If the boundary of the region R_1 is convex, then D_1 lies exterior to R_1. Suppose R has the property that whenever two distinct points A' and A'' of B are given, then R contains all points of the arc $A'OA''$ of the circle through O, A' and A''; under these circumstances D lies exterior to R.

If the region R_1 has an axis of symmetry, then D_1 lies on that axis. If the region R has an axis of symmetry S on which O lies, then D also lies on S, and (Theorem 2) is so situated that the circle through O and D orthogonal to S must either cut B or separate two components of B. If the region R is symmetric (anallagmatic) in a circle S on which O lies, then D also lies on S; the circle through O and D orthogonal to S must either cut B or separate two components of B.

Theorems 1 and 2 have application to the study of coefficients of the mapping function in a smooth conformal map. Let the function

$$w_1 = F(z) \equiv \frac{1}{z} + b_0 + b_1 z + b_2 z^2 + \dots \tag{1}$$

map the region $|z| < 1$ smoothly (schlicht) onto the region R_1 of Theorem 1 in the w_1-plane. Then[5] D_1 is the point $w_1 = b_0$. Let the function

$$w = \frac{1}{w_1} = f(z) \equiv z + a_2 z^2 + a_3 z^3 + \dots \tag{2}$$

map R_1 and $|z| < 1$ smoothly onto the region R of Theorem 2 in the w-plane; we do not assume that the point at infinity is not an interior point of R, so $f(z)$ must be meromorphic but not necessarily analytic throughout the region $|z| < 1$. The point at infinity is an interior point of R when and only when $w_1 = 0$ lies interior to R_1.

From equations (1) and (2) we find $a_2 = -b_0$, and for the region R of course $w = 1/b_0$ is the point D corresponding to the point $O : w = 0$.

It is an obvious consequence of (1) that at least one point of B_1 satisfies the relation $|w_1 - b_0| \geqq 1$, so at least one point of B satisfies the relation $\left|\frac{1}{w} + a_2\right| \geqq 1$.

It is a consequence of Theorem 1 that B_1 is cut by the line through $w_1 = b_0$ perpendicular to the line joining $w_1 = b_0$ to the origin $w_1 = 0$. Consequently, under the conditions of Theorem 2, the boundary B is cut by the circle $\left|w + \frac{1}{2a_2}\right| = \frac{1}{2|a_2|}$. In particular we have

$$\frac{1}{|a_2|} \geqq \text{distance from } O \text{ to } B.$$

We add two further remarks, applications of Theorem 2 to the study of the smooth conformal map $w = f(z)$ of the interior of $T: |z| = 1$ in the z-plane onto the region R of the w-plane.

Each coaxial family of circles K through two points z_1 and z_2 of the z-plane with $|z_1| < 1$, corresponds to a set of curves in R whose osculating circles at the point $w = f(z_1)$ form a coaxial family; each of these osculating circles cuts the image in R of every circle which lies interior to T, which separates z_1 from T, and which is orthogonal to the family K.

Let Q be either a circle which lies completely interior to R, or an analytic arc or curve each of whose osculating circles lies interior to R, or more generally an analytic arc or curve in R whose osculating circle at no point O (considered variable) passes through the corresponding point D of Theorem 2; then the image of Q in the z-plane is an arc or curve none of whose osculating circles is orthogonal to T. This remark is analogous to Carathéodory's remark that if R is convex, every line segment interior to R has as its image in the z-plane an arc each of whose osculating circles cuts T.[6]

[1] See, for instance, Study, E., *Konforme Abbildung einfach zusammenhängender Bereiche* (Leipzig, 1913); Peschl, E., *Math. Ann.*, **106**, 574–594 (1932); Walsh, J. L., *Amer. Math. Monthly*, **42**, 1–17 (1935).

[2] Walsh, loc. cit., §8.

[3] Walsh, loc. cit., Theorem 11.

[4] *Jour. de l'École polytechnique*, **28**, 1–33 (1879).

[5] *Math. Zeit.*, **3**, 78–86 (1919). Compare also Pólya-Szegö, *Aufgaben und Lehrsätze* (Berlin, 1925), II, p. 24.

[6] Whether or not R is convex, every line segment in the w-plane through the point $w = f(0)$ has as its image in the z-plane a curve whose osculating circle at the point $z = 0$ cuts the circle $|z| = 1/2$.

IRIDOPHYCUS IN THE NORTHERN HEMISPHERE

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In a previous preliminary paper (*Proc. Nat. Acad. Sci.*, **22**, 469–473, 1936), we gave our reasons for discarding the name "*Iridaea*" Bory, for replacing it with the new designation *Iridophycus*, and gave a list of the South American species arranged under four subgenera with a key to these species. This paper was followed by another (now in press) giving detailed descriptions and a comprehensive historical account of the species

reported from the Southern Hemisphere. These studies were in the nature of preparation for the study and interpretation of the species of the genus as represented in the Northern Hemisphere. We are presenting in this paper a similar preliminary report on our findings in this latter field up to date, hoping to follow up soon with a paper giving a more complete and detailed account with adequate illustrations.

The brief diagnoses and the following key treat of all the species of *Iridophycus* known to us in the Northern Hemisphere, all of which are to be referred to the subgenus *Euiridaea* J. Ag.

Iridophycus Agardhianum (J. Ag.) nom. nov.

Iridaea minor J. Ag., *in* Act. Holm., 86, 1849 (non Endlicher, Gen. Pl., Suppl. III, 30, 1843; non Kuetz., Tab. Phyc., 17:2, pl. 3 *d*, *e*, 1867).

Iridophycus cordatum (Turn.) comb. nov.

Fucus cordatus Turner, Hist. Fuc., 2: pl. 116, 1809.

Iridophycus cornucopiae (Post. et Rupr.) comb. nov.

Iridaea cornucopiae Post. and Rupr., Ill. Alg., p. 18, pl. 38, fig. *b*, 1840.

Iridophycus heterocarpum (Post. et Rupr.) comb. nov.

Iridaea heterocarpa Post. and Rupr., Ill. Alg., p. 18, 1840.

Iridophycus parvulum (Kjellm.) comb. nov.

Iridaea laminarioides var. *parvula* Kjellm., Om Beringhaf. Algfl. p. 31, 1889.

Iridophycus coriaceum sp. nov.

Frondibus plerumque amplis, usque ad 1.25 M. longis, 3 dm. latis, et 1 mm. crassis, vulgo late lanceolatis, integris, coriaceis, exsiccatis fere atris; cellulis medullaribus non aperte anastomosantibus, forma magnitudine variabilibus, usque ad 25μ diam.; cellulis subcorticalibus subsphericis, sparsis; corticibus angustissimis, filamentis anticlinis 3–4 cellularibus; cystocarpiis magnis, profunde in medulla positis; ramulis tetrasporangiiferis accessoriis numerosissimis et in medulla a latere quoque continuis.

Extending from Neah Bay, Washington, to the coast of central California. Type locality, Cypress Point, Monterey County, California. Type, Herb. Univ. Calif., Nos. 296,589 ♀, 296,600 + and 276,269 ♂.

Iridophycus splendens sp. nov.

Frondibus usque ad 9 dm. altis, plerumque angustis prae altitudine, usque ad 0.5 mm. crassis, lanceolatis, integris aut plus minusve in 2–4

lobis profunde divisis, violaceopurpureis usque ad exsiccatis rubro-purpureis; cellulis medullaribus non aperte anastomosantibus, e cylindricis usque ad fere sphericis, usque ad 10μ diam.; filamentis corticalibus anticlinis 4–6 cellularibus; cystocarpiis 1–2 mm. diam. exigue protuberantibus; soris tetrasporangiiferis numerosissimis, parvis et distinctis.

Coasts of central and southern California. Type locality, Carmel. Type sheets, Herb. Univ. Calif., Nos. 539,564, 539,565 and 539,566.

Iridophycus furcatum sp. nov.

Frondibus tenuibus sed rigidis, 8–15 cm. altis, plerumque ad apophysem in 2 planis orbicularibus lobis divisis; stipitibus distinctis; apophysibus comparate longis angustisque; e laeto- usque ad atropurpureis colore variantibus; cellulis medullaribus non aperte anastomosantibus, 3–6μ diam. et 4–8-plo longioribus; cellulis subcorticalibus ellipsoideis usque ad sphericis; filamentis corticalibus anticlinis 7–10-cellularibus; cystocarpiis sparsis, magnitudine variabilibus.

Sitka, Alaska, the type and only known locality. Type, Herb. Univ. Calif., No. 543,957.

Iridophycus flaccidum sp. nov.

Frondibus angustis prae altitudine, usque ad 1.4 M. altis, 8–20 cm. latis, usque ad 400μ crassis, comparate mollibus flaccidisque; stipitibus curtissimis; apophysibus cuneatis, brevibus latisque; exsiccatis infero atro violaceis, supero laetioribus; filamentis medullaribus angustissimis, libere aperteque anastomosantibus; cellulis subcorticalibus arachnoideis; filamentis corticalibus anticlinis 5–7 cellularibus, iis inferis sphericis usque ad cylindricis in iis superis; cystocarpiis comparate parvis et copiosis; soris tetrasporangiiferis copiosis, stricte delimitatis, ramellis tetrasporangiiferis interjacentibus egentibus.

Coast of central California. Type locality, Carmel. Type, Herb. Univ. Calif., Nos. 539,561 ♀, 547,654 + and 547,655 ♂.

Iridophycus lineare sp. nov.

Frondibus lineari-lanceolatis, marginibus plerumque crispatis, usque ad 1 M. longis, 3.5–7 cm. latis, integris aut in 2-paucis lobis usque ad apophysem divisis, purpureo-violaceis; stipitibus distinctis, 2–5 cm. longis; filamentis medullaribus non aperte anastomosantibus cellulis forma magnitudineque variabilibus compositis, usque ad 15μ diam. et 2–5-plo longioribus; cellulis subcorticalibus subsphericis; filamentis corticalibus anticlinis 4–5-cellularibus.

Growing in dense tufts along the coast of central California. Type locality, Carmel. Type, Herb. Univ. Calif., Nos. 507,637 ♀, 507,582 ♂ and 266,510 +

Iridophycus Parksii sp. nov.

Frondibus diminutivis, 2.5–4 cm. altis, rigidis; stipitibus curtis, in apophysibus conspicuis canaliculatisque mergentibus; laminis bi-trifurcatis, plerumque orbiculatolobatis; filamentis medullaribus aperte anastomosantibus, 2–4μ diam.; cellulis subcorticalibus arachnoideis; filamentis corticalibus anticlinis 5–6 cellularibus.

Growing in the upper littoral belt, near Trinidad, Humboldt County, California. Type, Herb. Univ. Calif., No. 507,492.

Iridophycus Reediae sp. nov.

Frondibus coriaceis, lanceolatis, 30–35 cm. altis, basi 3–5 cm. latis, atro-purpureo-violaceis; filamentis medullaribus parce anastomosantibus, cellulis forma magnitudineque variabilibus compositis, sphericis usque ad cylindricis, 5–35μ diam., in filamentis anticlinis corticalibus 4–6 cellularibus directe mergentibus.

Bushnell's Beach, San Luis Obispo County, California. Type, Herb. Univ. Calif., No. 392,651.

Iridophycus sanguineum sp. nov.

Frondibus usque ad 40 cm. longis et 20 cm. latis, plerumque integris, colore sanguinis bovis; filamentis medullaribus non aperte anastomosantibus, cellulis forma magnitudineque variabilibus, subsphericis usque ad cylindricis et usque ad 25μ diam.; filamentis corticalibus anticlinis 3–4-cellularibus.

Growing in the sublittoral belt, along the coast of central California. Type locality, Duxbury Reef, Marin County. Type, Herb. Univ. Calif., Nos. 507,503 ♀ and 507,504♂.

Iridophycus sinicola sp. nov.

Frondibus forma magnitudine maxime variabilibus, 8–50 cm. longis, 4–15 cm. latis, orbicularibus usque ad lanceolatis, tenuibus flaccidisque, integris usque ad profunde 2–3-furcatis, purpureis, iridescentibus; cellulis medullaribus 5–8μ diam., aperte anastomosantibus plerumque cylindricis; filamentis corticalibus anticlinis 5–6-cellularibus; cystocarpiis copiosis, parvis, in medulla profunde positis, super latera frondis utraque protuberantibus.

In the lower littoral belt, San Francisco Bay, California. Type, Herb. Univ. Calif., Nos. 510,685 ♀ and 510,684 +.

Iridophycus Whidbeyanum sp. nov.

Frondibus typicis orbicularibus, subcoriaceis, colore sanguinis bovis, 20–35 cm. altis; stipitibus curtissimis; apophysibus inconspicuis, apice partim in 2-pluribus lobis divisis; filamentis medullaribus non aperte

anastomosantibus, cellulis curtis, usque ad 12μ diam.; cellulis subcorticalibus subsphericis; filamentis corticalibus anticlinis 3–5-curtocellularibus; cystocarpiis parvis, plerumque marginalibus; ramulis accessoriis tetrasporangiiferis inter soros continuis.

Sublittoral belt, west coast of Whidbey Island, Washington. Type, Herb. Univ. Calif., Nos. 132,749 ♀, 94,380 ♂ and 94,378 +.

Iridophycus fulgens sp. nov.

Frondibus magnitudine mediis, latis prae altitudine, integris aut partim apice furcatis, basi cuneatis usque ad subcordatis, nitente iridescentibus; filamentis medullaribus non aperte anastomosantibus, cellulis forma magnitudine magnopere variabilibus, usque ad 16μ diam. et 2–3-plo longioribus; cellulis subcorticalibus arachnoideis; filamentis corticalibus anticlinis 5–6-cellularibus, cellulis subcylindricis; ramulis accessoriis tetrasporangiiferis copiosis et inter soros continuis.

Central and northern coasts of California. Type locality near Trinidad, Humboldt County. Type, Herb. Univ. Calif., No. 453,970.

Tentative key to the boreal species of *Iridophycus:*

1. Diminutive, less than 5 cm. high; stipe short, merging into a conspicuous, canaliculate apophysis; blade di-tri-chotomous..... .....1. *I. Parksii*
1. Fronds of medium size, 5–25 cm. high; stipe and apophysis variable; blade entire to variously lobed or divided at the apophysis.. 2
1. Fronds large, 25 cm. to over 1 meter long..... 7
   2. Fronds chiefly broad in proportion to length..... 3
   2. Fronds chiefly narrow in proportion to length, often branched and with marginal pinnules on the apophysis and blade.... .....2. *I. Agardhianum*
3. Tetrasporic sori numerous and for the most part confluent; fronds thin and membranaceous.....3. *I. parvulum*
3. Tetrasporic sori not confluent; fronds extremely variable in shape and thickness..... 4
   4. Fronds somewhat cornucopioid, the lower part being canaliculate.....4. *I. cornucopiae*
   4. Fronds plane for the most part throughout..... 5
5. Fronds for the most part once or twice divided at the apophysis; blade orbicular, membranaceous.....5. *I. furcatum*
5. Fronds entire or more or less deeply and variously lobed but very rarely divided at the apophysis..... 6
   6. Cystocarps relatively very large, up to 3 mm. diam., but variable in size even on the same plant..6. *I. heterocarpum*

6. Cystocarps of moderate size; fronds thin, membranaceous... ....7. *I. sinicola*
7. Fronds for the most part broader than long.....8. *I. Whidbeyanum*
7. Fronds usually from 2 to many times as long as broad..... 8
8. Fronds linear, many times longer than broad, mostly 5–10 cm. wide..... 9
8. Fronds broader..... 10
9. Stipe conspicuous, 2–5 cm. long, merging into a cuneate apophysis; medulla composed of a network of filaments 3–6μ wide, with cells many times as long as wide.....9. *I. lineare*
9. Stipe and apophysis shorter; medulla composed of cells up to 25μ diam., and 2–5 times as long as broad.....10. *I. Reediae*
10. Fronds up to 1.5 mm. thick, coriaceous, cartilaginous on drying; dark red to brownish at maturity, very dark on drying..... .....11. *I. coriaceum*
10. Fronds thinner, more or less iridescent when fresh..... 11
11. Fronds short and relatively broad for the most part, with the base decidedly cordate.....12. *I. cordatum*
11. Fronds relatively short and broad, mostly with cuneate base, brilliantly iridescent.....13. *I. fulgens*
11. Fronds usually decidedly longer than broad, for the most part with cuneate base..... 12
12. Fronds up to 2.5 times as long as broad; ox-blood red in color.....14. *I. sanguineum*
12. Fronds for the most part over 2.5 times as long as broad; color violet or purplish..... 13
13. Fronds somewhat coriaceous, more or less purplish and very highly iridescent when fresh.....15. *I. splendens*
13. Fronds thin and flaccid, violet color below.....16. *I. flaccidum*

EFFECT OF AUXIN ON CHLORELLA VULGARIS

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The greater part of experimental work with auxin has been done on higher plants.[1] Nothing is yet known about its action on lower organisms such as algae. In two recent papers[2,3] Leonian reported that "auxin" promotes the growth of some algae and the fungus *Phytophthora cactorum*.

Unfortunately Leonian used extracts from pea and algae. It is not at all certain that the active principle was auxin.

In the present study pure heteroauxin (β-indole-acetic acid) was used. The experimental material was *Chlorella vulgaris* from a pure culture that has been grown in the laboratory for more than 10 years by Dr. R. Emerson. Prior to the experiment the alga was replated on agar and the single-cell colonies were isolated and cultured. The cells from a single clone were used for inocula in all the subsequent experiments. The individuals of the clone showed a normal distribution of cell size.

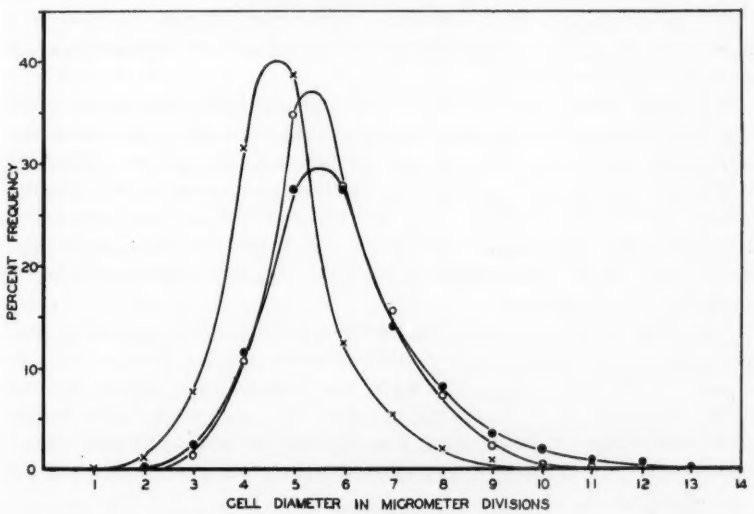

FIGURE 1

Variation of cell diameters in cultures. ×—control (without auxin), ○—0.01 mg./cc. heteroauxin, ●—0.04 mg./cc. heteroauxin.

The medium was prepared, as described by Emerson,[4] in culture flasks and sterilized in an autoclave. A stock solution of 20 mg. β-indole-acetic acid (Merck) in 100 cc. of water was made, sterilized separately and added to the media in different amounts. The osmotic concentration in all the media was about the same. One cc. of the mother culture (containing 2–3.5 mm.^{3} cells) was inoculated into each culture. The culture flasks were placed in a bath of running water and lighted from below by 5–7 60-watt bulbs. A constant current of 5 per cent carbon dioxide in air was passed through the flasks. Experiments lasted 2, 3 or 10 weeks. At the end of an experiment samples were taken from each culture and examined under the microscope. The diameters of about 1000 cells were measured by means of an eyepiece micrometer. To avoid personal errors in the

measurement only thin slide preparations were used so that all the cells came in view at about the same focus, and measurement was made on every cell in the field, which was moved by a mechanical stage.

The average cell diameters found in 2 experiments are given below.

CONC. OF HETEROAUXIN MG./CC.	AVERAGE DIAMETER OF CELLS IN μ EXPT. 1 (2 WEEKS)	EXPT. 2 (3 WEEKS)
0	5.99 ± 0.042	6.13 ± 0.034
0.001	8.07 ± 0.054	... ...
0.01	7.97 ± 0.049	7.13 ± 0.036
0.04	7.36 ± 0.051	7.50 ± 0.047

The range of variation of the cell diameters is shown in figure 1. It is evident from these results that auxin distinctly promotes cell enlargement in algae as it does in higher plants.

The total growth of the cultures as determined by centrifuging or by dry weight determinations showed considerable variation. No conclusive differences were found between the controls and the cultures containing 0.001–0.01 mg./cc. heteroauxin. At high concentrations of heteroauxin, injurious effects were evident: the cultures grew little and the cells had a reddish brown rather than a green color. They had very little chlorophyll and a very small photosynthetic activity. Further experiments along these lines are in progress.

The auxin in the cultures disappeared rapidly in the course of the experiments. In 2 weeks about $^1/_2$ of the original amount disappeared. In 3 weeks 80–90% was gone. No auxin was detected in the media and the $CHCl_3$ cell extracts of the control cultures. The alga seems to be unable to produce auxin by itself; or if it does, the amount is so small as to escape detection by the ordinary Avena method without concentration as in the case of Valonia.[5]

[1] F. W. Went, *Bot. Rev.*, **1**, 162–182 (1935).
[2] L. H. Leonian, *Jour. Agr., Res.*, **51**, 277–286 (1935).
[3] L. H. Leonian, *Bot. Gaz.*, **97**, 854–859 (1936).
[4] R. Emerson, *Jour. Gen. Physiol.*, **12**, 609–622 (1929).
[5] H. G. Van der Weij, *Proc. K. Akad. Wetensch., Amsterdam*, **36**, 759–760 (1933).

ON THE ORIGIN OF INTERSTELLAR RADIO DISTURBANCES

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Dr. Karl G. Jansky[1,2] of the Bell Telephone Laboratories has investigated electromagnetic disturbances at a wave-length of 14.6 meters by means of a rotating unidirectional antenna array, together with an automatic device for recording signal strength. He has definitely shown that continuous disturbances, highly directional in character, arise from an extra-terrestrial source fixed in space and approximately coincident with the galactic center in Sagittarius. The position determined by him is at $17^h 30^m$, $-20°$, with uncertainties not exceeding $\pm 30^m$ and $\pm 30°$, respectively. Jansky has suggested[2] that the radiations may arise from (1) the stars, or (2) secondary radiations induced by them in the earth's atmosphere or (3) thermal agitation of charged particles at high temperatures in interstellar space.

Jansky states that the maximum horizontal component of the continuous radiation never exceeds 0.39 microvolts per meter per kilocycle band width, which is equivalent to an intensity of 4.0×10^{-13} ergs/sec./cm.^{2}/kc. bd. An examination of Jansky's published data suggests that this intensity is an upper limit and that a lower limit for the total intensity penetrating the earth's atmosphere is about one-tenth this value or 4.0×10^{-14} ergs/sec./cm.^{2}/kc. bd. The absorption produced in the ionic layer of the earth's atmosphere at this wave-length is unknown but probably small.

The possibility of a stellar origin for these radiations is excluded by the non-appearance of a solar effect in the received intensities. The absence of the solar term seems also to exclude an origin in secondary radiations produced in the earth's atmosphere. Furthermore, it seems difficult to conceive of a suitable origin for such secondary radiations. The most probable alternative process appears to be one related to Jansky's third suggestion which has not yet been discussed. The fact that the disturbances were observed over a range of two meters in wave-length suggests the existence of a continuum of radiation rather than any atomic or molecular resonance phenomena.

Black-body radiation from sources at very low temperatures provides such a continuous band source. We shall investigate the possible intensity of such radiation from interstellar sources. On the present model of the galactic system,[3] the central mass is located at a distance of ten thousand parsecs and contains 1.3×10^{11} solar masses. If the average star has a mass and luminosity equal to those of the sun, the total luminosity of the central mass is 5×10^{44} ergs/sec. In the galactic plane there exists a nar-

row absorbing band of matter having a total extinction of approximately 0.5 magnitudes in a direction normal to the plane of the system. If we assume that absorbing material exists also in the galactic center and that it consists of particles capable of absorbing and emitting radiation as black bodies, we find that the particles will attain a temperature of approximately 10°K. It is interesting to calculate the maximum amount of radiation at 14.6 meters wave-length that may be received at the earth's distance from the galactic center.

It will be assumed that 15 per cent of the total radiation of the stars is absorbed by the obscuring material in the galactic center, an amount comparable to the absorption in the neighborhood of the sun. The total radiation from the absorbing particles is then 7.5×10^{43} ergs/sec. and may be assumed to have the black-body distribution corresponding to 10°K. The amount of radiation received at the earth in the 14.6 meters wave-length band is 4.4×10^{-20} ergs/sec./cm.^{2}/kc. bd. At the same wave-length the radiation received from the sun is 4.3×10^{-20} ergs/sec./cm.^{2}/kc. bd. It is clear that the solar effect could not have appeared in Jansky's measures, and also that the simple assumptions above will not account for the received intensities, which are 10^6 times greater. A simple integration shows that the total radiation from the rest of the galaxy within the cone of sensitivity of the antenna is only of the order of 5 per cent of the radiation from the galactic center.

We shall show that the extreme concentration of matter and radiation toward the center of the galactic system may cause the emission of a much greater quantity of long-wave radiation than is calculated above. If the absorbing material is similarly concentrated toward the center of the galaxy, and consists of small particles ($\approx 10^{-2}$ cm.) its opacity will be very high for wave-lengths shorter than 10^{-1} cm., the region embracing the energy maxima of the stellar and low temperature emissions. The energy density in the galactic nucleus will be considerably increased, resulting in an increased temperature and emission for the interstellar particles. The low opacity in the radio wave-lengths, arising from the small relative size of the particles, will selectively permit the escape of these longer wave-lengths. The problem of the radiative equilibrium in such a system is mathematically equivalent to that for an extended stellar atmosphere.

In order to radiate at 14.6 meters a particle must possess a natural mode of vibration of frequency 2×10^7/sec. The smallest particle that can maintain a standing wave of this frequency has a diameter equal to the velocity of sound divided by the frequency. For meteoric material this minimum diameter is $2a = 1.5 \times 10^{-2}$. The absorption by a particle (for light $\lambda < a$) is πa^2. The absorption per unit mass is $3/4ad$, where d is the density. Therefore we shall adopt the particle radius $a = 7.5 \times 10^{-3}$ cm. as compatible with the radiation of 14.6 meters waves and as

giving the maximum absorption per unit mass. We shall assume that the mass of the dark material is $M = 5 \times 10^{43}$ gm., approximately one-fifth of the total mass of the galactic nucleus, and that it is contained in a sphere of radius $R = 1500$ parsecs. There will be 9.5×10^{48} such particles, with a total surface area of 6.7×10^{45} cm.^{2}. The mean space density is 1.2×10^{-22} gm./cm.^{3} The absorption coefficient in the galactic nucleus for $\lambda < a$, is $13.6^m/kps$.

The fraction of the total black-body radiation in a given wave-length band ($\lambda \gg \lambda$ max.) is:

$$0.45 \times \lambda^{-4} \times T^{-3} \times d\lambda. \qquad (1)$$

The total emission from the small particles is $\sigma T^4 \times$ total surface. The radiation received at the earth from the small particles, neglecting absorption, in any wave-length band ($\lambda \gg \lambda$ max.), is:

$$1.45 \times 10^{-5} \times \lambda^{-4} \times d\lambda \times T. \qquad (2a)$$

At 14.6 meters wave-length this radiation is

$$2.2 \times 10^{-19} \times T^\circ \text{ ergs/sec./cm.}^2\text{/kc. bd./degree}, \qquad (2b)$$

where T° is the effective temperature of the particles.

For the purpose of the present investigation the galactic nucleus may be viewed as a gigantic star in which radiation and gas pressures are negligible and in which the opacity and energy sources are distributed according to the space density. In view of the high opacity all the emitted starlight will assume the energy distribution corresponding to the energy density of interstellar space. Consequently, the well-known principles of local thermodynamic equilibrium can be applied. We shall use the methods and notations of Chandrasekhar's development[4] of the Eddington-Milne solutions. Such a galactic nucleus actually viewed from the outside in visual light will appear as a diffuse, poorly resolved mass of rather low central condensation and surface brightness. The visual light will escape only from the outer regions. Some such condition may be the origin of the essential irresolvability and low observed luminosity of the central regions of external galaxies.

The conventional equation for radiative transfer, allowing for the sphericity of the equidensity surfaces, is:

$$\cos\theta \frac{\partial I}{\partial r} - \frac{\sin\theta}{r} \frac{\partial I}{\partial \theta} = -\rho(kI - kB - \epsilon), \qquad (3)$$

where θ is the angle between the normal and the line of sight; r is the radius; I is the total intensity per unit area; ρ is the space density of the absorbing material; k is the mass absorption coefficient (assumed constant over

the range of wave-lengths of maximum intensity); B is the integrated Planck function; $4\pi\epsilon$ is the energy generated per unit mass of absorbing material (assuming the solar value to hold for all the stellar energy sources). It is assumed that the stellar sources of energy are distributed along with the absorbing material according to its density ρ, which is taken to be of the form:

$$\rho = \rho_0/r^n. \tag{4}$$

Under the equilibrium conditions assumed, the equations (3) and (4) become:

$$\frac{d}{dr}(r^2F) = 4\,\rho_0\epsilon r^{(2-n)}, \tag{5}$$

where πF is the net outward flux. The integral is:

$$F = 4\,\frac{\rho_0\epsilon}{3-n}\,r^{(1-n)}, \tag{6}$$

the integration constant vanishing under the conditions assumed. Utilizing the Eddington type of approximation as developed by Chandrasekhar,[4] the final solution for B takes the following forms:

$$n = 0, \quad B = {}^1/_2k\rho_0^2\epsilon(R^2 - r^2) + {}^1/_2F_R \tag{7a}$$

$$n = 1, \quad B = {}^3/_2k\rho_0^2\epsilon \log\frac{R}{r} + {}^1/_2F_R \tag{7b}$$

$$n = 2, \quad B = {}^3/_2k\rho_0^2\epsilon\left(\frac{1}{r^2} - \frac{1}{R^2}\right) + {}^1/_2F_R \tag{7c}$$

In these equations M is the mass of the absorbing material in the galactic center; R is the radius of the center; $F_R = \dfrac{\epsilon M}{\pi R^2}$ is the net outward flux at the surface; and $\rho_0 = \dfrac{(3-n)M}{4\pi R^{(3-n)}}$. We wish to determine the mean temperature, $\bar{T}$. The temperature at any point, T, is given by:

$$\sigma T^4 = \pi B \tag{8}$$

and the mean temperature by:

$$\bar{T} = \frac{\int_0^R \rho r^2 T\,dr}{\int_0^R \rho r^2\,dr} \tag{9a}$$

$$\bar{T} = (3 - n)\int_0^1 \left(\frac{r}{R}\right)^{(2-n)} T d\left(\frac{r}{R}\right). \qquad (9b)$$

Since the fourth root of B enters into this integral, the evaluation is made more simply by numerical integration.

The numerical data adopted previously provide the following constants:

$R = 4.62 \times 10^{21}$ cm. $\quad \epsilon = 0.634$ ergs/sec./gm.
$M = 5.00 \times 10^{43}$ gm. $\quad k = 33.3$ cm.^{2}/gm.
$F_R = 0.473$ ergs/sec./cm.^{2}

The computed mean temperatures, and the intensity of radiation received at the earth from the galactic center at 14.6 meters wave-length, are given as a function of n (the degree of central concentration of the nucleus) in the following table:

n	T	RADIATION RECEIVED AT 14.6 METERS
0	16.5°	3.6×10^{-18} ergs/sec./cm.^{2}/kc. bd.
1	17.9°	3.9×10^{-18} ergs/sec./cm.^{2}/kc. bd.
2	30.0°	6.6×10^{-18} ergs/sec./cm.^{2}/kc. bd.

Comparing these values for the radiation at 14.6 meters with Jansky's measures we see that black-body radiation in the most favorable case fails by a factor of 10^4 to account for the observations. Keeping the total mass and energy generation constant, the radiating surface will be increased by $\left(\frac{a_0}{a}\right)$ and the mean temperature by $\left(\frac{a_0}{a}\right)^{1/4}$ (see equations (7) and (9)), if the particle radius is decreased from a_0 to a. Such a process cannot be carried far because of the difficulty of obtaining long-wave radiations from such small particles and because of the incredibly high resultant opacity.

In view of the failure of black-body radiation to account for Jansky's observations quantitatively it is particularly necessary to investigate more thoroughly the actual dependence of the received intensity on wave-length, a problem which is now being attacked by one of the authors.

[1] Jansky, K. G., *Proc. Inst. Rad. Eng.*, **21**, 1387 (1933).
[2] Jansky, K. G., *Proc. Inst. Rad. Eng.*, **23**, 1158 (1935).
[3] Plaskett, J. S., *Publ. Dom. Ast. Obs.*, **5**, 242 (1936).
[4] Chandrasekhar, S., *Rus. A. J.*, **11**, 550 (1934).

NEW EVIDENCE PERTAINING TO PUERTO RICAN PREHISTORY

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In 1934, the first Peabody Museum expedition to Puerto Rico excavated the only two clearly stratified midden sites that have been discovered in the West Indies. In each of these sites, near Ponce and Luquillo, Dr. F. G. Rainey discovered a stratum containing crab remains and painted pottery underneath a stratum which contained sea shells and incised pottery. Dr. Rainey called the material found in the lower layers the "Crab Culture," and the material in the upper layers, the "Shell Culture." Since he also found both cultural stages in a third site near Isabela on the opposite end of the island, he concluded that they were general throughout Puerto Rico, and that everywhere the earlier Crab Culture horizon had been followed by a Shell Culture horizon.[1] In 1935, on a second expedition for the museum, Dr. Rainey found fragments of some of the carved stone objects which are so numerous in the surface collections of Puerto Rican archaeology, such as "collarstones," in the Shell Culture deposits of the Luquillo site, and therefore attributed this stonework to the later, or Shell Culture, horizon.[2]

The third Peabody Museum expedition to Puerto Rico last summer made an archaeological survey of the eastern end of the island, with the assistance of funds supplied by the Graduate School of Yale University and by the University of Puerto Rico. During the ten weeks devoted to this survey, I located, described and mapped sixty-nine sites in the eastern half of Puerto Rico,[3] and excavated test pits four meters square in twelve of the more important of these sites. I dug test pits in refuse deposits only, i.e., in shell middens along the coast and in pottery deposits inland. However, I was able to locate and excavate refuse deposits associated with most other manifestations of Puerto Rican archaeology, including cave sites, petroglyphs and the so-called "ball courts," "stonecollars" and "three-pointed stones."

New data of three kinds resulted from these excavations. In the first place, two of the sites excavated, Ceiba No. 1 and Guayama, as well as ten others surveyed, appeared to contain no pottery. Clear evidence of human occupation, in the form of charcoal, split and burned shells, and animal bones, was found in each of the sites excavated. However, the only artifacts obtained were a number of chisel-like conch-shell objects of a type rarely found in pottery sites, and these occurred in the Ceiba No. 1 site only. As a result, it is not possible to determine either the chronological position or the cultural affinities of these non-pottery sites, though it is

tentatively suggested that they are earlier than the pottery-bearing sites. Two facts favor this suggestion. In the first place, non-pottery sites seem to have been older than pottery sites in Cuba,[4] Haiti,[5] the Dominican Republic[6] and the Virgin Islands.[7] Secondly, the Ceiba No. 1 non-pottery site is only 350 meters away from a pottery site. If these two sites were contemporaneous, there should have been contact and trade of pottery between their inhabitants.

The second discovery made last summer was a stratigraphical sequence containing a transition from Crab to Shell Culture material. This sequence was observed in the two deepest sites excavated, one near Trujillo Alto and the other in the municipality of Cidra. At the bottom of the Trujillo Alto site, 2.75 meters below the surface, were typical Crab Culture sherds. A comparatively large number of these sherds were decorated, predominately with red and white painted designs. There was a gradual change in the nature of the sherds in the intermediate levels of this site, and at its top very few of the sherds were decorated, and these only with red-painted bands. In the lower levels of the Cidra site, 2.50 meters deep, the sherds were of the same general nature as those found at the top of the Trujillo Alto site. There was again a gradual change in the intermediate levels, and at the top of the site the number of decorated sherds was much larger. These sherds, however, were decorated characteristically with incised designs of different types than the painted designs. They were typical Shell Culture sherds.

This transition from the Crab Culture to the Shell Culture may be interpreted as follows. When the Trujillo Alto site (at least the part excavated) was first settled, the inhabitants decorated their pottery primarily with designs painted in two colors, red and white. Gradually, they abandoned two-color painting in favor of simple red painting, and at the same time they left more and more of their pottery undecorated. At the time when the Trujillo Alto site was being abandoned and the Cidra site was being settled, the fashion of leaving the pottery undecorated was at the peak of its popularity. Eventually, after the Trujillo Alto site had been abandoned, this fashion passed the peak and began to decline in popularity. The reason was that a new technique of pottery decoration, incision, came into use and began to be employed more and more frequently. The later inhabitants of the Cidra site incised proportionately as much pottery as the original inhabitants of the Trujillo Alto site had painted.

The time of habitation of the Trujillo Alto and Cidra sites may be divided roughly into three periods, corresponding to the times when it was most fashionable to paint the pottery, to leave it undecorated and to incise it. These three periods may be called the Crab, Intermediate and Shell Periods, respectively.

The other sites excavated can be dated relative to these periods. On figure 1 are tentatively suggested the chronological positions of each of the sites in the eastern half of Puerto Rico excavated last summer, as well as of the three sites excavated by Dr. Rainey during the two preceding summers (Ponce, Luquillo and Isabela). Each site has been named for the muncipality in which it occurs. The length of the line for each site indicates the probable length of time during which the excavated portion of each site was inhabited and is based on the relative depth of deposit in the pit excavated in each site. The position of the line for each site indicates approximately the age of the excavated portion of the site and is based on the relative frequency of incised, undecorated and painted sherds observed during the excavation of the site.[8]

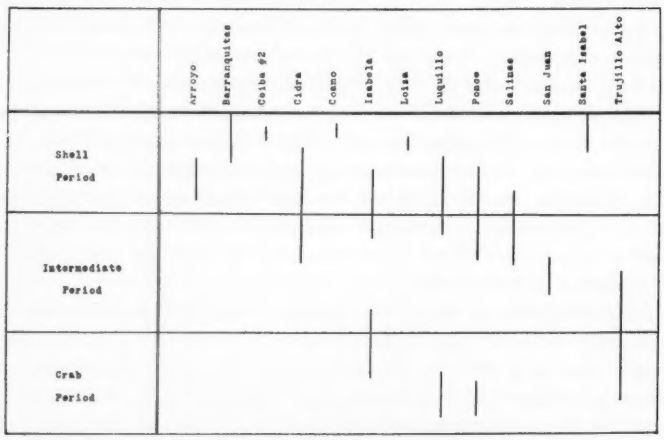

FIGURE 1

Probable chronological position of excavated portions of sites.

The third evidence obtained last summer concerned the relation between the Crab-to-Shell Periods sequence and other known manifestations of Puerto Rican archaeology, including inland refuse sites, ball courts, petroglyphs and cave sites. Previous excavations in village refuse heaps have been limited to sites on the coastal plains. Last summer, refuse deposits in three mountain village sites, Barranquitas, Cidra and Coamo, were excavated. The cultural material obtained from these sites resembles that obtained from the coastal sites, with the exception that only the Coamo site contained an appreciable amount of crab and shell fish remains. On the other hand, two coastal sites, Loiza and Trujillo Alto, likewise lacked sea food remains. It would seem, therefore, that the customs of eating crab and sea shell food were limited to the regions near the coast where

those animals were readily obtainable. Of the sites excavated last summer in which there were sea food remnants, those inhabited during the Crab Period contained only crab jaws, and those inhabited during the Shell Period contained mainly sea shells, and only occasionally a few crab jaws. Four of the five shell midden sites inhabited during the Intermediate Period (all except the San Juan site) contained a mixture of crab jaws and sea shells.[9] It may be concluded, therefore, that in the regions where sea food was obtainable crabs but not shell fish were eaten during the Crab Period, both were consumed during the Intermediate Period and shell fish were almost exclusively eaten during the Shell Period.

Ball courts were found associated with the three inland sites excavated last summer, Barranquitas, Cidra and Coamo, all of which were inhabited during the Shell Period. Petroglyphs were definitely associated with two Shell Period sites, Barranquitas and Coamo, and possibly also with a third, the Ceiba No. 2 site. The one cave site excavated, at Loiza, appears to have been occupied during the Shell Period. Ball courts, petroglyphs and cave sites, therefore, must have been in use during the Shell Period.

The new evidence obtained last summer necessitates a revision of the reconstruction of Puerto Rican prehistory suggested by Dr. Rainey. In order to explain the differences between the Crab and Shell Period strata in the Luquillo and Ponce sites, Dr. Rainey postulated two separate "periods of migration" from South America through the Lesser Antilles into Puerto Rico.[10] The discovery of a transition between the Crab and Shell Cultures in the Trujillo Alto and Cidra sites now makes this hypothesis unlikely. The differences between the Crab and Shell Period strata in the Luquillo and Ponce sites must have been the result of a hiatus in the habitation of the excavated portions of those sites during the Intermediate Period, as indicated on figure 1, rather than of the arrival of an alien people. The two sites may have been completely abandoned toward the close of the Crab Period, and then resettled by a local people toward the end of the Intermediate Period. On the other hand, since less than half of the deposits in each of the sites were excavated, the original inhabitants may have moved only to the unexcavated portion of each site during the Intermediate Period, and then moved back to the excavated areas later. The latter explanation is probable for the Ponce site, since I observed transition-like material in the part of that site not excavated.

In either case, it appears likely that a single group of people lived in Puerto Rico during the Crab, Transition and Shell Periods. The similarities between the culture of these Indians and the culture of Indians in neighboring islands can be ascribed to trade, interchange of ideas, occasional infiltrations of small groups of people and perhaps some intermarriage, rather than to sudden large migrations from South America.

With this revision of Dr. Rainey's conclusions, it becomes possible to

correlate the results obtained by Peabody Museum expeditions in Puerto Rico and Haiti with the results obtained by Hatt from excavations in the Virgin Islands and Santo Domingo. Hatt discovered two phases of culture in the Virgin Islands analogous to the Crab and Shell Period cultures in Puerto Rico. The earlier cultural phase was similar to the culture of the Lesser Antilles, but the later phase resembled the culture of Santo Domingo. Hatt therefore postulated two "cultural movements," the first one westward from the Lesser Antilles into the Virgin Islands during our Crab Period, and the second eastward from the Dominican Republic through Puerto Rico into the Virgin Islands during our Shell Period.[11] Since it is possible to demonstrate detailed resemblances between the Shell Period culture in Puerto Rico and the later Carrier phase of culture in Haiti,[12] it now appears likely that in Puerto Rico, as in the Virgin Islands, there were cultural influences from Hispaniola during the Shell Period in contrast to the cultural influences from the Lesser Antilles during the Crab Period.

To conclude, there are traces in Puerto Rico of an early period when pottery was not in use. The time when pottery was used can be divided into three periods, the Crab Period when the pottery was characteristically painted, the Intermediate Period when it was usually left undecorated and the Shell Period when it was characteristically incised. During these periods, a single descent of people, perhaps the ancestors of the historic Borinquén Indians, seem to have lived in Puerto Rico. As time passed, these people gradually changed many of their food-eating and pottery-making customs, at first often as the result of influences from the inhabitants of the Lesser Antilles, and later during the Shell Period more often because of influences from the natives of Hispaniola and Cuba. During the Shell Period, these people are known to have constructed ball courts within their villages, carved petroglyphs, frequented caves and made stonecollars.[13]

[1] Rainey, F. G., "A New Prehistoric Culture in Puerto Rico," *Proc. Nat. Acad. Sci.*, **21**, 12–16 (1935).

[2] Rainey, F. G., "A New Prehistoric Culture in Haiti," *Proc. Nat. Acad. Sci.*, **22**, 7 (1936).

[3] I am indebted to a number of people for information concerning these sites. Dr. F. G. Rainey kindly supplied me with his revision of a list of sites compiled by Dr. Lothrop. I received much help from inhabitants of the region surveyed. The bulk of my information, however, came from Puerto Rican archaelogists, particularly Mr. Adolfo de Hostos, Mr. Benigno Fernández-García, Mr. R. L. Junghanns and Dr. J. L. Montalvo-Guenard.

[4] Harrington, M. R., *Cuba Before Columbus*, Part I, Vols. I-II, Indian Notes and Monographs, Museum of the Am. Indian, Heye Found., New York, p. 406 (1921).

[5] Rainey, F. G., op. cit., pp. 4–8 (1936).

[6] Krieger, H. W., "Aboriginal Indian Pottery of the Dominican Republic," *Bull. 156, U. S. National Museum*, Washington, 30 (1931).

[7] Hatt, Gudmund, "Archaeology of the Virgin Islands," *Proc. XXI Int. Congress of Americanists, First Part, the Hague,* 31 (1924).

[8] The data for the three sites excavated by Dr. Rainey have been taken from statistics in his *Puerto Rican Archaeology,* a MS. in the Yale University Library.

[9] The data for the Luquillo, Isabela and Ponce sites are from Dr. Rainey's MS.

[10] Rainey, F. G., op. cit., p. 7 (1936).

[11] Hatt, Gudmund, op. cit., p. 40.

[12] For definition of this type, see Rainey, p. 6 (1936).

[13] A brief note on the excavations appeared in *Antiquity,* **2,** No. 3, 227–228 (1936).